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Cover photo: *Reginaia ebenus* (Lea, 1831), the type species of *Reginaia* Campbell and Lydeard, 2012. The genera of Pleurobemini (Bivalvia: Unionidae: Ambleminae) (Campbell and Lydeard, 19–38). Photo courtesy of David Campbell.

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Molecular systematics of *Fusconaia* (Bivalvia: Unionidae: Ambleminae)

David C. Campbell[1] and Charles Lydeard[2]

[1]Paleontological Research Institution, 1259 Trumansburg Road, Ithaca, New York 14850, U.S.A.

[2]The National Science Foundation, 4201 Wilson Boulevard, Arlington, Virginia 22230, U.S.A.

Correspondence, D. C. Campbell: pleuronaia@gmail.com

Abstract: The genus *Fusconaia* Simpson, 1900, as currently recognized, includes ~12 species in the tribe Pleurobemini. Two species are federally listed and several more are imperiled in part or all of their ranges; one species is probably extinct. However, classification at the species and genus level has been problematic, and it is unknown whether imperiled populations represent merely local ecophenotypic variations or endemic species. To provide additional evidence on the systematics of this group and to help establish conservation priorities, we sequenced two mitochondrial genes for all available species of *Fusconaia* as well as representatives of other genera of Pleurobemini and several outgroups. Both *cox1* and *nad1* provided well-resolved phylogenies. Some putative species show little molecular differentiation, supporting their synonymization. In particular, *Fusconaia flava* (Rafinesque, 1820), *F. cerina* (Conrad, 1838), and the easternmost populations previously assigned to *F. askewi* (Marsh, 1896) are not differentiated by our data. Although the majority of *Fusconaia* places in a well-supported clade that includes *F. flava*, the type species, others do not. "*Fusconaia*" *barnesiana* (Lea, 1838), the type of *Pleuronaia* Frierson, 1927, places with "*Lexingtonia*" *dollabelloides* (Lea, 1840) and "*Pleurobema*" *gibberum* (Lea, 1838). "*Fusconaia*" *ebenus* (Lea, 1831) and "*F.*" *rotulata* (Wright, 1899) form a distinct clade outside of Pleurobemini. "*Fusconaia*" *succissa* (Lea, 1852) is assigned to the *pustulosa* group of *Quadrula* (subgenus *Rotundaria*), along with *Quincuncina infucata* (Conrad, 1834). Conversely, the type species of *Quincuncina*, *Quincuncina burkei* Walker, 1922, is assigned to *Fusconaia*. Populations in the Ozark region assigned to *F. flava* and populations in the Suwannee River system assigned to *Quincuncina infucata* probably deserve species-level recognition.

Key words: freshwater mussels, nomenclature, *Reginaia, Rotundaria*, Ambleminae

The genus *Fusconaia* Simpson, 1900, as currently recognized, includes about 12 species in eastern North America, ranging from central Texas to the James River drainage in Virginia and north through the Mississippi drainage to the Great Lakes and Hudson Bay systems (Burch 1975). *Fusconaia* is characterized by tetragenous brooding, in contrast to the rest of Pleurobemini; a distinct angle on the posterior slope (in most species); and a smooth, oval to quadrate shell, becoming trigonal in some populations.

Several uncertainties exist regarding classification in the genus. At the tribe level, *Fusconaia* was grouped with *Quadrula* Rafinesque, 1820 rather than with Pleurobemini in the classifications of Simpson (1900, 1914) and Heard and Guckert (1971) due to its generally tetragenous brooding. Molecular data, however, consistently place *Fusconaia* in Pleurobemini (*e.g.*, Davis and Fuller 1981, Campbell *et al.* 2005). At the species level, the number and identity of taxa outside of the main Mississippi basin remains unclear. In particular, the number of species in the southwestern portion of its range (Ozark region south to western Louisiana and Texas) and in the Mobile basin are debated (Vidrine 1993, Howells *et al.* 1996, Burdick and White 2007, Graf and Cummings 2007, Williams *et al.* 2008).

Genetic data provide an independent line of evidence for taxonomy, complementing detailed morphological and anatomical studies. A few species of Pleurobemini have been included in almost every previous molecular study on Ambleminae, and Lydeard *et al.* (2000), Campbell *et al.* (2005), and Burdick and White (2007) included several species of *Fusconaia*. These analyses have highlighted problematic taxa, such as *Fusconaia ebenus* (Lea, 1831), *Obovaria/Fusconaia rotulata* (Wright, 1899), *Fusconaia/Pleuronaia barnesiana* (Lea, 1838), and *Fusconaia/Quadrula succissa* (Lea, 1852), which seem to place outside of *Fusconaia*, and *Quincuncina burkei* Walker, 1922, which places in *Fusconaia* (unlike other *Quincuncina*). We sought to build on these studies by including more species and more data for a comprehensive look at the genus.

MATERIALS AND METHODS

Taxa were selected to represent a wide range of species of Pleurobemini, including the type species of genera when available, and as many species of *Fusconaia* as possible. Outgroups were selected to represent the other tribes of Ambleminae. We obtained material for all of the currently recognized extant species of *Fusconaia* except *F. lananensis* (Frierson, 1901) as well as representatives of the other genera of Pleurobemini. Although *F. lananensis* has been recognized by most publications, in practice differentiation of *Fusconaia* species in Texas is quite difficult. The type localities for *F. askewi* (Marsh, 1896) and *F. lananensis* are in adjacent drainages (Sabine and Neches, respectively) and the species may be conspecific. We also generated extensive data for a population of *Fusconaia*, including forms with different soft part coloration, in an effort to identify mussels from a site in the Hiwassee River sampled as part of a North Carolina Department of Transportation project. The new data also include several additional specimens from the *F. flava* (Rafinesque, 1820) complex from various localities throughout its range. Representatives of other tribes were used as outgroups. We sequenced portions of two mitochondrial genes that had proven useful for species-level studies on unionids: cytochrome oxidase I (*cox1*) (Roe and Lydeard 1998) and NADH dehydrogenase I (*nad1*) (Buhay *et al.* 2002, Serb *et al.* 2003). We also tried nuclear ITS1 sequences. Although it has worked for some other unionids, ITS1 failed to give clear resolution within Pleurobemini (Campbell *et al.* 2008). We generated sixty-one new sequences (Appendices 1–2).

DNA was extracted from fresh, frozen, or ethanol-preserved specimens using standard CTAB and chloroform-isoamyl alcohol protocols (Winnepenninckx *et al.* 1993). The ITS1 region and portions of the *cox1*, and *nad1* genes were amplified. Primers for *cox1* were 5′-GTTCCACAAATCAT AAGGATATTGG-3′ and 5′-TACACCTCAGGGTGACC AAAAA ACCA-3′, adapted from Folmer *et al.* (1994); primers for *nadh1* were 5′-TGGCAGAAAAGTGCATCAGATTTA AGC-3′ and 5′-GCTATTAGTAGGTCGTATCG-3′ (Buhay *et al.* 2002, Serb and Lydeard 2003); and primers for ITS1 were 5'-AAAAAGCTTCCGTAGGTGAACCTGCG-3' and 5'-AGCTTGCTGCGTTCTTCATCG-3' (King *et al.* 1999). PCR cycles were: 92 °C 2 min; 92 °C 40 s, 40 °C 40 s, 72 °C 90 s 5× 92 °C 40 s, 50 °C 40 s, 72 °C 90 s 25×; 72 °C 10 min; hold 4 °C. PCR products were purified using Qiagen QIAquick PCR purification kits. Cycle sequencing used ABI Big Dye 3.1 Terminator kits with thermal cycle parameters of 1 °C per second ramp speed, starting with 1 min at 96 °C followed by 26 cycles of 96 °C for 10 s, 49 °C for 5 s, and 60 °C for 4 min, then 10 min at 60 °C and hold at 4 °C. Cycle sequencing products were purified with sephadex columns or Qiagen DyeEx kits and then run on an automated sequencer.

The results for each strand were compared and aligned using BioEdit (Hall 1999). Sequences were analyzed using PAUP* 4.10 (Swofford 1998) and MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). Maximum parsimony trees were generated for *cox1* and *nad1* data independently as well as a combined analysis using all species represented by data for both genes. Identical sequences were eliminated from the analyses as they cause problems for parsimony and MrBayes. Except for the identical haplotype reported by Burdick and White (2007) from both *Fusconaia cerina* and *F. flava* and one haplotype found in putative *F. askewi* (as initially identified) and *F. flava*, all haplotypes were confined to a single species. Composite *F. subrotunda* (Lea, 1831) sequences were used when there were multiple identical sequences. These sets of sequences were *F. subrotunda*10, 18, 19, 25, 29, 35 and *F. subrotunda* 4, 5, 15, 20, 22, 23, 24, 26, 27, 28, 32, 33, 34, 50. A partition-homogeneity test was run in PAUP* (PILD of Dowton and Austin 2002; note their discussion of the limits of these tests). The *P* value was 0.189, so the two mitochondrial genes were concatenated for further analysis. Because of the large number of very similar sequences for the *F. flava* complex (many of which were partial *cox1* sequences) and *F. subrotunda*, separate analyses were performed including all non-identical sequences for those two groups, while only a representative set of the most complete sequences for these taxa were included in the larger analyses. Maximum parsimony analyses used 500 random replicates, hold = 10, swap = TBR. Bootstrap analyses used 500 replicates, each using a random parsimony search of 10 replicates. MrModeltest 2.2 (Nylander 2004) favored a GTR+I+G model, which was implemented in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) with 2,000,000 generations and 8 chains; revmat, shape, pinvar, and statefreq were unlinked.

RESULTS

ITS1 supports Pleurobemini as a clade and a few species-level subclades, but otherwise failed to provide resolution within Pleurobemini. It does support the exclusion of *Fusconaia ebenus* from Pleurobemini. In contrast, mitochondrial genes provided relatively good resolution. Results for *cox1*, *nad1*, and for the combined mitochondrial analysis were in strong agreement. We found strong support for Pleurobemini and *Fusconaia sensu stricto*; however, several putative *Fusconaia* placed outside of *Fusconaia* (Figs. 1–6). Within *Fusconaia*, several subclades received strong support. Single gene trees (Figs. 3–4) had somewhat lower support values, reflecting the shorter length and several partial sequences. In particular, support for clades from *cox1* was weak, reflecting the fact that, within the standard Folmer region (Folmer *et al.* 1994), Burdick and White (2007) used approximately the first and last 1/3, whereas Lydeard *et al.* (2000) used approximately the last 2/3. The topology within *Fusconaia* varied little between analyses, but the relationships between genus-level clades in Pleurobemini vary and are not well-resolved. The analyses of *cox1* data for *F. flava* and *F. subrotunda* likewise support similar topologies in *Fusconaia* (Figs. 5–6). In all analyses, species with multiple specimens are strongly supported as monophyletic, apart from the *F. flava-cerina-*"*askewi*" complex, and unresolved relationships within the "*F.*" *ebenus* (Lea, 1831)-"*F.*" *rotulata* (Wright, 1899) and *F. burkei* (Walker, 1922)-*F. escambia* Clench and Turner, 1956 clades.

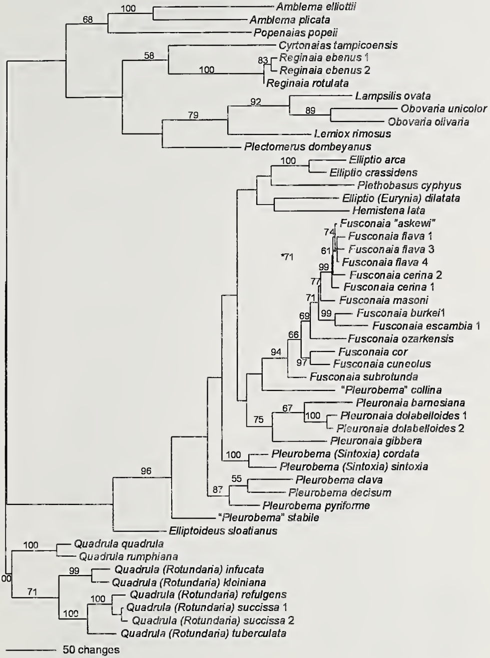

100
68
Amblema elliottii
Amblema plicata
Popenaias popeii
Cyrtonaias tampicoensis
58
83
Reginaia ebenus 1
100
Reginaia ebenus 2
Reginaia rotulata
Lampsilis ovata
92
89
Obovaria unicolor
79
Obovaria olivaria
Lemiox rimosus
Plectomerus dombeyanus
100
Elliptio arca
Elliptio crassidens
Plethobasus cyphyus
Elliptio (Eurynia) dilatata
Hemistena lata
Fusconaia "askewi"
74
Fusconaia flava 1
61
Fusconaia flava 3
*71
99
Fusconaia flava 4
Fusconaia cerina 2
77
Fusconaia cerina 1
71
Fusconaia masoni
99
Fusconaia burkei1
69
Fusconaia escambia 1
66
Fusconaia ozarkensis
94
Fusconaia cor
97
Fusconaia cuneolus
Fusconaia subrotunda
"Pleurobema" collina
67
Pleuronaia barnesiana
75
100
Pleuronaia dolabelloides 1
Pleuronaia dolabelloides 2
Pleuronaia gibbera
100
Pleurobema (Sintoxia) cordata
96
Pleurobema (Sintoxia) sintoxia
55
Pleurobema clava
87
Pleurobema decisum
Pleurobema pyriforme
"Pleurobema" stabile
Elliptoideus sloatianus
100
Quadrula quadrula
Quadrula rumphiana
99
Quadrula (Rotundaria) infucata
100
Quadrula (Rotundaria) kleiniana
71
100
Quadrula (Rotundaria) refulgens
100
Quadrula (Rotundaria) succissa 1
Quadrula (Rotundaria) succissa 2
Quadrula (Rotundaria) tuberculata
50 changes

Figure 1. Phylogram of strict consensus maximum parsimony tree (3 trees, length = 2930, CI = 0.3205, RI = 0.5970), *cox1* and *nad1* data combined. Numbers on branches are bootstrap percentages. The * indicates where there is not room to put the 71% support number on the branch for the clade of *Fusconaia flava* 1, *F. flava* 3, and *F. flava* 44.

All *Fusconaia* from the Hiwassee River site were identified as *F. subrotunda*. No significant genetic differences were found between individuals with white versus salmon-colored soft parts. No specimens of *F. cor* (Conrad, 1834) or *F. cuneolus* (Lea, 1840) were found, but the molecular analyses did readily detect the specimens of *Pleurobema oviforme* (Conrad, 1834) and *Villosa iris* (*sensu lato*) (Lea, 1829) included among the tissue clips.

Within *Fusconaia*, most analyses identify *F. subrotunda* as the basally diverging taxon, followed by the *F. cor-cuneolus* clade, but this topology is only weakly supported. The remaining species of *Fusconaia* form a clade with generally poorly resolved internal relationships, suggesting relatively rapid radiation and wide dispersal. However, there is strong support for a clade of *F. escambia* and "*Quincuncina*" *burkei*, both from the drainages between the Mobile and Apalachicola systems. Likewise, there is strong support for the *F. flava-cerina* complex. Among species listed as *Fusconaia* or *Quincuncina* that placed outside of *Fusconaia*, "*Fusconaia*" *succissa* (Lea, 1852) appeared closely related to *Quadrula*

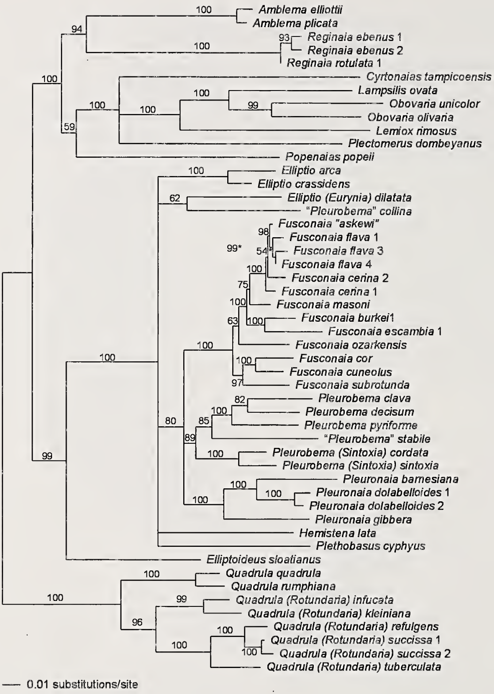
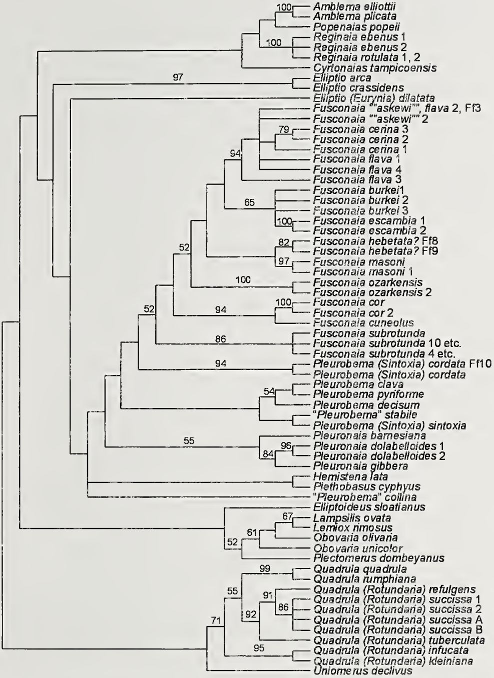

100
Amblema elliottii
Amblema plicata
94
93
Reginaia ebenus 1
100
Reginaia ebenus 2
Reginaia rotulata 1
100
Cyrtonaias tampicoensis
100
Lampsilis ovata
100
100
99
Obovaria unicolor
Obovaria olivaria
59
Lemiox rimosus
Plectomerus dombeyanus
Popenaias popeii
100
Elliptio arca
Elliptio crassidens
62
Elliptio (Eurynia) dilatata
"Pleurobema" collina
Fusconaia "askewi"
98
Fusconaia flava 1
99*
54
Fusconaia flava 3
Fusconaia flava 4
100
Fusconaia cerina 2
75
Fusconaia cerina 1
100
Fusconaia masoni
63
100
Fusconaia burkei1
Fusconaia escambia 1
100
Fusconaia ozarkensis
100
100
Fusconaia cor
Fusconaia cuneolus
97
Fusconaia subrotunda
82
Pleurobema clava
100
Pleurobema decisum
80
85
Pleurobema pyriforme
89
"Pleurobema" stabile
99
100
Pleurobema (Sintoxia) cordata
Pleurobema (Sintoxia) sintoxia
100
Pleuronaia barnesiana
100
Pleuronaia dolabelloides 1
100
Pleuronaia dolabelloides 2
Pleuronaia gibbera
Hemistena lata
Plethobasus cyphyus
Elliptoideus sloatianus
100
Quadrula quadrula
Quadrula rumphiana
100
99
Quadrula (Rotundaria) infucata
Quadrula (Rotundaria) kleiniana
96
100
Quadrula (Rotundaria) refulgens
Quadrula (Rotundaria) succissa 1
100
100
Quadrula (Rotundaria) succissa 2
Quadrula (Rotundaria) tuberculata
0.01 substitutions/site

Figure 2. Phylogram of majority rule consensus of trees from Bayesian analyses (burn1 = 13500; burn2 = 15600; final average standard deviation of split frequencies = 0.009274; mean ln likelihood = -13958.617; most likely tree ln likelihood = -13928.071), *cox1* and *nad1* data combined. Numbers are posterior probabilities. The * indicates where there is not room to put the 99% support number on the branch for the clade of *Fusconaia flava* 1, *F. flava* 3, and *F. flava* 44.

refulgens (Lea, 1868) (also supported as part of the same clade in Serb *et al.* 2003). "*Quincuncina*" *infucata* (Conrad, 1834) and "*Quincuncina*" *kleiniana* (Lea, 1852) are strongly supported as close relatives of each other, and are generally supported as more distant relatives of "*F.*" *succissa*. "*Fusconaia*" *ebenus* and "*F.*" *rotulata* appear closely related but are not strongly associated with any other sampled species. Finally, "*F.*" *barnesiana* (Lea, 1838) is closely related to "*Lexingtonia*" *dollabelloides* (Lea, 1840) and "*Pleurobema*" *gibberum* (Lea, 1838).

Intraspecific variation in *cox1* was generally low, with greater percent difference roughly correlated with taxonomic distance (Table 1). Some variation in the degree of difference may reflect the presence of partial sequences.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Amblema plicata
Popenaias popeii
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Reginaia ebenus 2
Reginaia rotulata 1, 2
Cyrtonaias tampicoensis
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Elliptio (Eurynia) dilatata
Fusconaia ""askewi"", flava 2, Ff3
Fusconaia ""askewi"" 2
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Fusconaia cerina 3
Fusconaia cerina 2
Fusconaia cerina 1
94
Fusconaia flava 1
Fusconaia flava 4
Fusconaia flava 3
Fusconaia burkei1
65
Fusconaia burkei 2
Fusconaia burkei 3
100
Fusconaia escambia 1
Fusconaia escambia 2
82
Fusconaia hebetata? Ff8
52
Fusconaia hebetata? Ff9
97
Fusconaia masoni
Fusconaia masoni 1
100
Fusconaia ozarkensis
Fusconaia ozarkensis 2
100
Fusconaia cor
52
94
Fusconaia cor 2
Fusconaia cuneolus
Fusconaia subrotunda
86
Fusconaia subrotunda 10 etc.
Fusconaia subrotunda 4 etc.
94
Pleurobema (Sintoxia) cordata Ff10
Pleurobema (Sintoxia) cordata
Pleurobema clava
54
Pleurobema pyriforme
Pleurobema decisum
"Pleurobema" stabile
Pleurobema (Sintoxia) sintoxia
Pleuronaia barnesiana
55
96
Pleuronaia dolabelloides 1
84
Pleuronaia dolabelloides 2
Pleuronaia gibbera
Hemistena lata
Plethobasus cyphyus
"Pleurobema" collina
Elliptoideus sloatianus
67
Lampsilis ovata
Lemiox rimosus
61
Obovaria olivaria
52
Obovaria unicolor
Plectomerus dombeyanus
99
Quadrula quadrula
Quadrula rumphiana
55
Quadrula (Rotundaria) refulgens
91
Quadrula (Rotundaria) succissa 1
86
Quadrula (Rotundaria) succissa 2
92
71
Quadrula (Rotundaria) succissa A
Quadrula (Rotundaria) succissa B
Quadrula (Rotundaria) tuberculata
95
Quadrula (Rotundaria) infucata
Quadrula (Rotundaria) kleiniana
Uniomerus declivus

Figure 3. Strict consensus of 233 maximum parsimony trees (length = 1225, CI = 0.3306, RI = 0.6308), *cox1* data. Numbers on branches are bootstrap percentages. "4 etc." indicates the combined 4, 5, 15, 20, 22, 23, 24, 26, 27, 28, 32, 33, 34, and 50 sequences (identical except for different amounts of missing data at the ends) and "10 etc." indicates the combined 10, 18, 19, 25, 29, and 35 sequences (identical except for different amounts of missing data at the ends). Multiple names on a single branch indicate multiple identical alleles. There was also 56% bootstrap support for a clade of *Fusconaia burkei* 1 and 2, not supported in the strict consensus.

DISCUSSION

The presence of a few well-supported clades in a background of generally poor resolution for ITS1 suggests that it has undergone occasional purifying selection but more often has freely diverged, possibly affected by ancestral polymorphisms or hybridization. The ITS1 sequence for *Quadrula* (*Rotundaria*) *tuberculata* (Rafinesque, 1820) includes a partial duplication of the gene region (a longer duplication was observed in *Strophitus undulatus* (Say, 1816) [Campbell, pers. obs.; this matches the anomalously long product in White *et al.* 1996]). As the duplicate regions are not identical, future evolution that reduces the sequence to normal length could generate a mosaic of descendant sequences in different

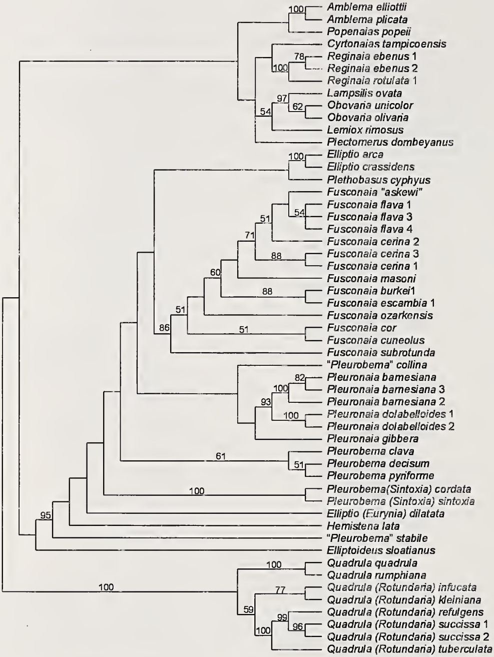
100
Amblema elliottii
Amblema plicata
Popenaias popeii
Cyrtonaias tampicoensis
78
Reginaia ebenus 1
100
Reginaia ebenus 2
Reginaia rotulata 1
97
Lampsilis ovata
62
Obovaria unicolor
54
Obovaria olivaria
Lemiox rimosus
Plectomerus dombeyanus
100
Elliptio arca
Elliptio crassidens
Plethobasus cyphyus
Fusconaia "askewi"
Fusconaia flava 1
54
51
Fusconaia flava 3
Fusconaia flava 4
71
Fusconaia cerina 2
88
Fusconaia cerina 3
Fusconaia cerina 1
60
Fusconaia masoni
88
Fusconaia burkei1
Fusconaia escambia 1
51
Fusconaia ozarkensis
51
86
Fusconaia cor
Fusconaia cuneolus
Fusconaia subrotunda
"Pleurobema" collina
82
Pleuronaia barnesiana
100
Pleuronaia barnesiana 3
93
Pleuronaia barnesiana 2
100
Pleuronaia dolabelloides 1
Pleuronaia dolabelloides 2
Pleuronaia gibbera
61
Pleurobema clava
51
Pleurobema decisum
Pleurobema pyriforme
100
Pleurobema(Sintoxia) cordata
Pleurobema (Sintoxia) sintoxia
95
Elliptio (Eurynia) dilatata
Hemistena lata
"Pleurobema" stabile
Elliptoideus sloatianus
100
Quadrula quadrula
Quadrula rumphiana
100
77
Quadrula (Rotundaria) infucata
Quadrula (Rotundaria) kleiniana
59
99
Quadrula (Rotundaria) refulgens
96
Quadrula (Rotundaria) succissa 1
100
Quadrula (Rotundaria) succissa 2
Quadrula (Rotundaria) tuberculata

Figure 4. Strict consensus of the 6 maximum parsimony trees (length = 1810, CI = 0.3094, RI = 0.6906), *nad1* data. Numbers on branches are bootstrap percentages. There was 54% bootstrap support for a clade of *Fusconaia "askewi"*, *F. cerina*, *F. flava*, *F. burkei*, and *F. escambia*, not supported in the strict consensus.

populations. Such an event in the past could account for the confusing mix of sequences observed today in Pleurobemini.

Most currently recognized species were supported in the present results. However, the *Fusconaia flava* species complex (*F. flava* and *F. cerina*) was not resolved as mutually monophyletic. Putative *F. askewi* specimens from western Louisiana in the Calcasieu River, just west of the lower Mississippi system, were not genetically differentiated from this complex. In fact, one *cox1* allele found in putative *F. cerina* and one found in putative *F. askewi* were identical to alleles found in *F. flava*. However, ongoing study, including genetic data, on Pleurobemini from Texas shows that *F. askewi* and *F. lananensis* from the Neches and Sabine drainage in Texas and Louisiana represent a distinct species from the *flava* complex (Burlakova *et al.*, unpublished). The lack of genetic differentiation between *F. flava* and *F. cerina* in the present study (also in Burdick and White 2007) suggests that they are conspecific, although study with nuclear markers is desirable (Jones *et al.* 2006). The *cox1* analyses (Figs. 3, 5) place all the *F. cerina* sequences in a clade, but also include one *F. flava*

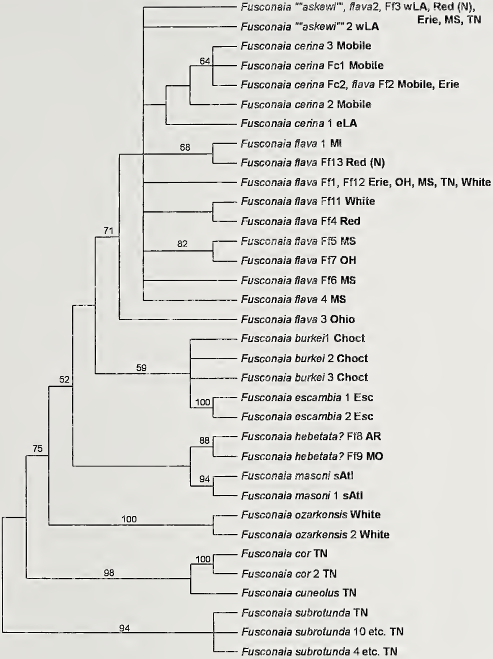
Fusconaia ""askewi"", flava2, Ff3 wLA, Red (N), Erie, MS, TN
Fusconaia ""askewi"" 2 wLA
Fusconaia cerina 3 Mobile
64
Fusconaia cerina Fc1 Mobile
Fusconaia cerina Fc2, flava Ff2 Mobile, Erie
Fusconaia cerina 2 Mobile
Fusconaia cerina 1 eLA
68
Fusconaia flava 1 MI
Fusconaia flava Ff13 Red (N)
Fusconaia flava Ff1, Ff12 Erie, OH, MS, TN, White
Fusconaia flava Ff11 White
Fusconaia flava Ff4 Red
71
82
Fusconaia flava Ff5 MS
Fusconaia flava Ff7 OH
Fusconaia flava Ff6 MS
Fusconaia flava 4 MS
Fusconaia flava 3 Ohio
Fusconaia burkei1 Choct
59
Fusconaia burkei 2 Choct
52
Fusconaia burkei 3 Choct
100
Fusconaia escambia 1 Esc
Fusconaia escambia 2 Esc
88
Fusconaia hebetata? Ff8 AR
75
Fusconaia hebetata? Ff9 MO
94
Fusconaia masoni sAtl
Fusconaia masoni 1 sAtl
100
Fusconaia ozarkensis White
Fusconaia ozarkensis 2 White
100
Fusconaia cor TN
98
Fusconaia cor 2 TN
Fusconaia cuneolus TN
Fusconaia subrotunda TN
94
Fusconaia subrotunda 10 etc. TN
Fusconaia subrotunda 4 etc. TN

Figure 5. Strict consensus of the 180 maximum parsimony trees (length = 205, CI = 0.7951, RI = 0.8547), *cox1* data for *Fusconaia flava-cerina* group. Numbers on branches are bootstrap percentages. "4 etc." indicates the combined 4, 5, 15, 20, 22, 23, 24, 26, 27, 28, 32, 33, 34, and 50 sequences (identical except for different amounts of missing data at the ends) and "10 etc." indicates the combined 10, 18, 19, 25, 29, and 35 sequences (identical except for different amounts of missing data at the ends). Multiple names on a branch indicate multiple identical alleles. There was 52% bootstrap support for a clade of *Fusconaia burkei*; 62% bootstrap support for a clade of *F. burkei* 1 and 2; and 55% bootstrap support for a clade of *F. subrotunda* 4 etc. and 10 etc.; not supported in the strict consensus. Bold labels indicate drainage or region (*i.e.*, this includes tributaries): **AR**, Arkansas River; **Choct**, Choctawhatchee River; **eLA**, eastern Louisiana; **Erie**, Lake Erie; **Esc**, Escambia River; **MI**, Lake Michigan; **MO**, Missouri River; **Mobile**, Mobile Basin; **MS**, Mississippi River (excluding Ohio River and Ozark region systems); **OH**, Ohio River (excluding Tennessee River system); **Red (N)**, Red River of the North; **Red**, Red River (Louisiana); **sAtl**, southern Atlantic (James River and south); **TN**, Tennessee River; **White**, White River (Arkansas, Missouri); **wLA**, western Louisiana.

sequence from a Lake Erie tributary, and the clade does not receive bootstrap support. The minimal sequence divergence within the *flava-cerina* clade provides little data for parsimony.

Several additional taxa, not recognized in Turgeon *et al.* (1998), may deserve recognition (in addition to the apparently extinct "*Fusconaia*" *apalachicola* Williams and Fradkin, 1999). Our results support previous studies that found

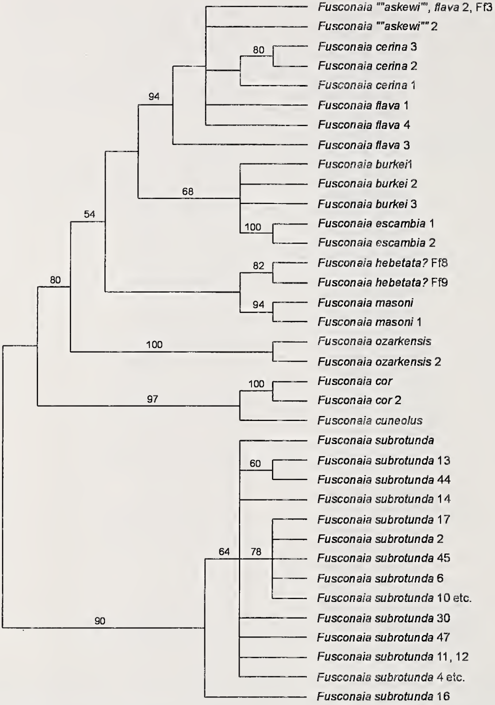
Fusconaia ""askewi"", flava 2, Ff3
Fusconaia ""askewi"" 2
80
Fusconaia cerina 3
Fusconaia cerina 2
Fusconaia cerina 1
94
Fusconaia flava 1
Fusconaia flava 4
Fusconaia flava 3
Fusconaia burkei1
68
Fusconaia burkei 2
Fusconaia burkei 3
54
100
Fusconaia escambia 1
Fusconaia escambia 2
82
Fusconaia hebetata? Ff8
80
Fusconaia hebetata? Ff9
94
Fusconaia masoni
Fusconaia masoni 1
100
Fusconaia ozarkensis
Fusconaia ozarkensis 2
100
Fusconaia cor
97
Fusconaia cor 2
Fusconaia cuneolus
Fusconaia subrotunda
60
Fusconaia subrotunda 13
Fusconaia subrotunda 44
Fusconaia subrotunda 14
Fusconaia subrotunda 17
Fusconaia subrotunda 2
64
78
Fusconaia subrotunda 45
Fusconaia subrotunda 6
Fusconaia subrotunda 10 etc.
90
Fusconaia subrotunda 30
Fusconaia subrotunda 47
Fusconaia subrotunda 11, 12
Fusconaia subrotunda 4 etc.
Fusconaia subrotunda 16

Figure 6. Strict consensus of the 82 maximum parsimony trees (length = 217, CI = 0.7972, RI = 0.8875), *cox1* data for *Fusconaia subrotunda*. Numbers on branches are bootstrap percentages. "4 etc." indicates the combined 4, 5, 15, 20, 22, 23, 24, 26, 27, 28, 32, 33, 34, and 50 sequences (identical except for different amounts of missing data at the ends) and "10 etc." indicates the combined 10, 18, 19, 25, 29, and 35 sequences (identical except for different amounts of missing data at the ends). Multiple names on a branch indicate multiple identical alleles. There was 57% bootstrap support for a clade of *F. burkei* 1 and 2, not supported in the strict consensus.

phylogenetically distinct forms. Lydeard *et al.* (2000) noted that the population of "*Quincuncina infucata*" from the Suwannee River system was phylogenetically distinct. Our results support distinguishing this taxon from true *infucata*. *Unio kleinianus* Lea, 1852 is available for this taxon. Burdick and White (2007) found distinctive haplotypes (their *Ff8* and *Ff9*) in putative *F. flava* from the Ozark region. Our additional *F. flava* complex samples strongly support identifying these samples as a presently unrecognized species. The present results also indicate that *Ff8* and *Ff9* are

Table 1. Percent sequence differences, *cox1*. All pairwise comparisons between the listed taxa were made. Thus, every *cox1* sequence for any species of *Fusconaia* as restricted herein was compared with every sequence for *Reginaia* Campbell and Lydeard, 2012 to generate the numbers in the first row

Comparison	Mean %	Max. %	Min. %
Fusconaia-Reginaia	11.99	16.59	10.52
Fusconaia-other Pleurobemini	7.64	16.21	3.46
Fusconaia subrotunda intraspecies	0.42	1.94	0.00
Fusconaia flava intraspecies	0.73	2.35	0.00
Fusconaia cerina intraspecies	1.23	2.87	0.20
Fusconaia "*askewi*" intraspecies	not applicable	0.17	0.17
Fusconaia flava-cerina-"*askewi*" interspecies	1.04	4.24	0.00
other interspecies within *Fusconaia*	4.63	14.36	1.76

not *F. ozarkensis* (Call, 1887). Other possible names for western *Fusconaia* include *Unio hebetatus* Conrad, 1854, from Missouri (no drainage information was given) and *Unio chunii* Lea, 1861, from the Trinity River at Dallas, Texas, in addition to *F. askewi* and *F. lananensis*. The other odd haplotype of Burdick and White (2007), *Ff10*, is assignable to "*Pleurobema*" *cordatum*. Graf and Cummings (2007) tentatively recognized *F. hebetata* (Conrad, 1854) from Missouri and *F. rubidula* (Frierson, 1905) from the Black Warrior drainage in Alabama as possibly valid, in addition to the species recognized in Turgeon *et al.* (1998). All Mobile Basin *Fusconaia* sampled placed with *F. cerina* or "*F.*" *ebenus*, suggesting that *F. rubidula* is a synonym of *F. cerina* (also in Williams *et al.* 2008), but *F. hebetata* may be appropriate for *Ff8* and *Ff9*. Williams *et al.* (2008) also reported an apparently undescribed, possibly extinct, species of *Fusconaia* from the Chattahoochee River, resembling true *Fusconaia* rather than "*F.*" *apalachicola*.

In contrast to general support for the species-level taxonomy, several species assigned to *Fusconaia* or its subjective synonyms *Quincuncina* and *Lexingtonia* evidently belong elsewhere. This result was also found in previous molecular studies such as Lydeard *et al.* (2000), Serb *et al.* (2003), and Campbell *et al.* (2005, 2008). Some even belong in other tribes. "*Fusconaia*" *succissa* should be transferred to Quadrulini, along with "*Quincuncina*" *infucata* and "*Quincuncina*" *kleiniana*, as also found by Lydeard *et al.* (2000), Serb *et al.* (2003), Campbell *et al.* (2005), and Williams *et al.* (2008). All four taxa are members of the *pustulosa* group in Quadrulini. Several genus- or subgenus-level names are available for this group, but the oldest is *Rotundaria* Rafinesque, 1820 (see Campbell and Lydeard 2012 for details).

"*Fusconaia*" *ebenus* and "*Obovaria*" or "*Fusconaia*" *rotulata* form a well-supported clade (as in Lydeard *et al.* 2000 and Campbell *et al.* 2005). "*Fusconaia*" *apalachicola* is known only from prehistoric middens and so was unavailable for DNA analysis; however, the shell morphology closely matches "*F.*" *ebenus* and "*F.*" *rotulata* in many aspects, supporting its assignment to the same group. The relationships of this clade to the rest of Ambleminae remain poorly resolved. A close relationship between these three was already suspected on morphological and anatomical grounds (Stansbery 1971, Williams and Butler 1994, Athearn 1998, Williams and Fradkin 1999, Williams *et al.* 2008). Although the oldest type designation for *Obovaria* Rafinesque, 1819 (in Herrmannsen 1847) would have made this the genus for the *ebenus* group, use of the genus *Obovaria* is well-established for the group of *Unio retusa* Lamarck, 1819 [*sic*; Lamarck used the feminine ending] (Bogan *et al.* 2006). Given the disruption this would cause for prevailing usage, the ICZN ruled to uphold general usage of *Obovaria* for *retusus* Lamarck and its relatives (ICZN 2008). All this leaves *ebenus* and its relatives without a genus, so Campbell and Lydeard (2012) propose the name *Reginaia*.

One other species generally assigned to *Fusconaia* belongs within Pleurobemini, but does not group with *Fusconaia*. "*Fusconaia*" *barnesiana*, "*Lexingtonia*" *dollabelloides*, and "*Pleurobema*" *gibberum* form one clade (Campbell *et al.* 2005). The genus *Pleuronaia* Frierson, 1927 is available for this group (Williams *et al.* 2008). The remaining *Fusconaia* species form a large clade, including not only the type of *Fusconaia* but also that of *Quincuncina*, *Quincuncina burkei*. Williams *et al.* (2008) report that molecular analyses of *Lexingtonia subplana* (Conrad, 1837), the type species of *Lexingtonia*, show that it is a synonym of *Fusconaia masoni* (Conrad, 1834). Our *F. masoni* data show that it is a member of *Fusconaia*, thus making *Lexingtonia* a synonym of *Fusconaia* and not appropriate for "*L.*" *dollabelloides*.

Most species of *Fusconaia* are geographically constrained, with the exception of the *F. flava* complex (Fig. 5). As *F. flava* has only recently reached the middle Tennessee River, following impoundment (Parmalee and Bogan 1998), this group probably dispersed between the Mobile Basin and the Mississippi Basin via the Pearl and Pascagoula Basins in Mississippi and east Louisiana rather than through the Tennessee Basin. *Reginaia ebenus*, in contrast, occurred historically in the middle Tennessee River up to Muscle Shoals (Williams *et al.* 2008) as well as in drainages between the Mobile Basin and the lower Mississippi River and could have dispersed between the Mobile Basin and the Mississippi system either via the Pearl and Pascagoula Basins or through the Tennessee Basin. No direct evidence indicates where either group originated, though the greater diversity and basal lineages of *Fusconaia* in the Tennessee-Cumberland-Ohio system may suggest an origin there. Conversely, the presence of sibling taxa in the Escambia and Apalachicola drainages weakly suggests that *Reginaia* has been in the Mobile Basin and vicinity a long time.

A few clades have clear geographic patterns. There is strong support for a clade of *Fusconaia escambia* and *F. burkei*, both from the drainages between the Mobile and Apalachicola systems. Likewise, the Cumberlandian *F. cor* and *F. cuneolus* are sister taxa. *Quadrula* (*Rotundaria*) *succissa* is most closely related to *Q.* (*R.*) *refulgens* and other species to the west (Serb *et al.* 2003) and more distantly to species the east such as *R. infucata* and *R. kleiniana*, matching the geographic relationships of *Pleurobema strodeanum* (Wright, 1898) (Campbell *et al.* 2008). Several unionids and other taxa show a major biogeographic break at the Apalachicola River (Avise 2000, Williams *et al.* 2008).

Summary

Fusconaia flava, F. cerina, and a population previously assigned to *F. askewi* (in the Calcasieu drainage, west Louisiana) are not mutually monophyletic based on mitochondrial DNA and may be conspecific. Populations currently assigned to *F. flava* from the northern and western Ozark region are genetically distinct from *F. flava* and *F. ozarkensis*. These may be attributable to *F. hebetata*, but the identity of that species is not clear. Apart from that, species assigned to *Fusconaia* generally appear well differentiated based on the molecular data, with low intrapopulation variation in *F. subrotunda* and fairly low interpopulation variation in *F. flava/cerina*. The type species of *Quincuncina* and *Lexingtonia* place within *Fusconaia*, whereas some species previously assigned to *Fusconaia* belong elsewhere. These include "*Fusconaia*" *barnesiana*, which is the type of *Pleuronaia* and is closely related to "*Lexingtonia*" *dollabelloides* and "*Pleurobema*" *gibberum* and some species that place outside of Pleurobemini. "*Fusconaia*" *ebenus* and "*Fusconaia/Obovaria*" *rotulata* are assigned to *Reginaia*; "*F.*" *apalachicola* is assigned here on conchological grounds. "*Fusconaia*" *succissa*, "*Quincuncina*" *infucata*, and "*Quincuncina*" *kleiniana* belong in Quadrulini.

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Appendix 1. Sequences used. =, this sequence was identical to another sequence and was not analyzed separately. *, new sequences.

Taxon	Source	Accession	Gene
Amblema elliottii (Lea, 1856)	Campbell *et al.* 2005	AY655086	*nad1*
Amblema elliottii	Campbell *et al.* 2005	AY654991	*cox1*
Amblema plicata (Say, 1817)	Serb *et al.* 2003	AY158796	*nad1*
Amblema plicata	Graf and Ó Foighil 2000	AF156512	*cox1*
Cyrtonaias tampicoensis (Lea, 1838)	Campbell *et al.* 2005	AY655090	*nad1*
Cyrtonaias tampicoensis	Bogan and Hoeh 2000	AF231749	*cox1*
Elliptio arca (Conrad, 1834)	Campbell *et al.* 2005	AY655093	*nad1*
Elliptio arca	Campbell *et al.* 2005	AY654995	*cox1*
Elliptio crassidens (Lamarck, 1819)	Campbell *et al.* 2008	DQ383428	*cox1*
Elliptio crassidens	Campbell *et al.* 2005	AY613788	*nad1*
Elliptoideus sloatianus (Lea, 1840)	Campbell *et al.* 2005	AY613790	*nad1*
Elliptoideus sloatianus	Campbell *et al.* 2005	AY613822	*cox1*
Eurynia dilatata (Rafinesque, 1820)	Graf and Ó Foighil 2000	AF156506	*cox1*
Eurynia dilatata	Campbell *et al.* 2008	DQ385872	*nad1*
Fusconaia "askewi" (Marsh, 1896)	UAM3392	*HM230411	*nad1*
Fusconaia "askewi" [sequence = *flava* 2]	UAM3392	*HM230367	*cox1*
Fusconaia "askewi" 2	UAM3395	*HM230366	*cox1*
Fusconaia burkei (Walker, 1922)	Serb *et al.* 2003	AY158793	*nad1*
Fusconaia burkei 2	Lydeard *et al.* 2000	AF232803	*cox1*
Fusconaia burkei 3	Lydeard *et al.* 2000	AF232802	*cox1*
Fusconaia burkei 1	Lydeard *et al.* 2000	AF232804	*cox1*
Fusconaia cerina 1 (Conrad, 1838)	UAM3376	*HM230368	*cox1*
Fusconaia cerina 1	Campbell *et al.* 2005	AY655095	*nad1*
Fusconaia cerina 2	Campbell *et al.* 2005	AY613823	*cox1*
Fusconaia cerina 2	Campbell *et al.* 2005	AY613792	*nad1*
Fusconaia cerina 3	Roe and Lydeard 1998	AF049522	*cox1*
Fusconaia cerina 3	UAM3235	*HM230412	*nad1*
Fusconaia cerina Fc1	Burdick and White 2007	DQ298537	*cox1*
Fusconaia cerina Fc2	Burdick and White 2007	DQ298538	*cox1*
Fusconaia cor (Conrad, 1834)	Campbell *et al.* 2005	AY655096	*nad1*
Fusconaia cor	Campbell *et al.* 2005	AY654997	*cox1*
Fusconaia cor 2	UAM2605	*HM230369	*cox1*
Fusconaia cuneolus (Lea, 1840)	Campbell *et al.* 2005	AY655097	*nad1*
Fusconaia cuneolus	Campbell *et al.* 2005	AY654998	*cox1*
Fusconaia escambia	UAM3403	*HM230350	ITS1
Fusconaia escambia 1 Clench and Turner, 1956	UAM3403	*HM230413	*nad1*
Fusconaia escambia 1	Lydeard *et al.* 2000	AF232816	*cox1*
Fusconaia escambia 2	Lydeard *et al.* 2000	AF232817	*cox1*
Fusconaia flava 1 (Rafinesque, 1820)	Graf and Ó Foighil 2000	AF156510	*cox1*
Fusconaia flava 1	Campbell *et al.* 2005	AY613793	*nad1*
Fusconaia flava 2	Serb *et al.* 2003	AY158781	*nad1*
Fusconaia flava 2 [= "*askewi*"]	Bogan and Hoeh 2000	AF231733	*cox1*
Fusconaia flava 3	Lydeard *et al.* 2000	AF232822	*cox1*
Fusconaia flava Ff1	Burdick and White 2007	DQ298524	*cox1*
Fusconaia flava Ff11	Burdick and White 2007	DQ298534	*cox1*
Fusconaia flava Ff12 [= *Ff1*]	Burdick and White 2007	DQ298535	*cox1*
Fusconaia flava Ff13	Burdick and White 2007	DQ298536	*cox1*
Fusconaia flava Ff2 [= *cerina Fc2*]	Burdick and White 2007	DQ298525	*cox1*
Fusconaia flava Ff3 [= "*askewi*"]	Burdick and White 2007	DQ298526	*cox1*
Fusconaia flava Ff4	Burdick and White 2007	DQ298527	*cox1*
Fusconaia flava Ff5	Burdick and White 2007	DQ298528	*cox1*
Fusconaia flava Ff6	Burdick and White 2007	DQ298529	*cox1*

Appendix 1. (Continued)

Taxon	Source	Accession	Gene
Fusconaia flava Ff7	Burdick and White 2007	DQ298530	*cox1*
Fusconaia flava 4	UAM2648	*HM230370	*cox1*
Fusconaia flava 4	UAM2648	*HM230414	*nad1*
Fusconaia hebetata? Ff8 (Conrad, 1854)	Burdick and White 2007	DQ298531	*cox1*
Fusconaia hebetata? Ff9	Burdick and White 2007	DQ298532	*cox1*
Fusconaia masoni (Conrad, 1834)	NCSM-H	*HM230415	*nad1*
Fusconaia masoni	NCSM-H	*HM230371	*cox1*
Fusconaia masoni 1	Morrison *et al.* 2005	EF619921	*cox1*
Fusconaia ozarkensis (Call, 1887)	UAM3501	*HM230416	*nad1*
Fusconaia ozarkensis	UAM3501	*HM230373	*cox1*
Fusconaia ozarkensis 2	UAM3500	*HM230372	*cox1*
Fusconaia subrotunda (Lea, 1831)	Campbell *et al.* 2005	AY613794	*nad1*
Fusconaia subrotunda	Campbell *et al.* 2005	AY613824	*cox1*
Fusconaia subrotunda 10 [= 10 etc.]	UAM3237	*HM230374	*cox1*
Fusconaia subrotunda 11	UAM3237	*HM230375	*cox1*
Fusconaia subrotunda 12 [= 11]	UAM3237	*HM230376	*cox1*
Fusconaia subrotunda 13	UAM3237	*HM230377	*cox1*
Fusconaia subrotunda 14	UAM3237	*HM230378	*cox1*
Fusconaia subrotunda 15 [= 4 etc.]	UAM3237	*HM230379	*cox1*
Fusconaia subrotunda 16	UAM3237	*HM230380	*cox1*
Fusconaia subrotunda 17	UAM3237	*HM230381	*cox1*
Fusconaia subrotunda 18 [= 10 etc.]	UAM3237	*HM230382	*cox1*
Fusconaia subrotunda 19 [= 10 etc.]	UAM3237	*HM230383	*cox1*
Fusconaia subrotunda 2	UAM3237	*HM230384	*cox1*
Fusconaia subrotunda 20 [= 4 etc.]	UAM3237	*HM230385	*cox1*
Fusconaia subrotunda 22 [= 4 etc.]	UAM3237	*HM230386	*cox1*
Fusconaia subrotunda 23 [= 4 etc.]	UA 20698	*HM230387	*cox1*
Fusconaia subrotunda 24 [= 4 etc.]	UAM3237	*HM230388	*cox1*
Fusconaia subrotunda 25 [= 10 etc.]	UAM3237	*HM230389	*cox1*
Fusconaia subrotunda 26 [= 4 etc.]	UAM3237	*HM230390	*cox1*
Fusconaia subrotunda 27 [= 4 etc.]	UAM3237	*HM230391	*cox1*
Fusconaia subrotunda 28 [= 4 etc.]	UA 20698	*HM230392	*cox1*
Fusconaia subrotunda 29 [= 10 etc.]	UAM3237	*HM230393	*cox1*
Fusconaia subrotunda 30	UAM3237	*HM230394	*cox1*
Fusconaia subrotunda 32 [= 4 etc.]	UAM3237	*HM230395	*cox1*
Fusconaia subrotunda 33 [= 4 etc.]	UAM3237	*HM230396	*cox1*
Fusconaia subrotunda 34 [= 4 etc.]	UAM3237	*HM230397	*cox1*
Fusconaia subrotunda 35 [= 10 etc.]	UAM3237	*HM230398	*cox1*
Fusconaia subrotunda 4 [= 4 etc.]	UAM3237	*HM230399	*cox1*
Fusconaia subrotunda 44	UAM3237	*HM230400	*cox1*
Fusconaia subrotunda 45	UA 20699	*HM230401	*cox1*
Fusconaia subrotunda 47	UA 20700	*HM230402	*cox1*
Fusconaia subrotunda 5 [= 4 etc.]	UAM3237	*HM230403	*cox1*
Fusconaia subrotunda 50 [= 4 etc.]	UA 20701	*HM230404	*cox1*
Fusconaia subrotunda 6	UAM3237	*HM230405	*cox1*
Hemistena lata (Rafinesque, 1820)	Campbell *et al.* 2005	AY613796	*nad1*
Hemistena lata	Campbell *et al.* 2005	AY613825	*cox1*
Lampsilis ovata (Say, 1817)	Roe *et al.* 2001	AF385111	*cox1*
Lampsilis ovata	Campbell *et al.* 2005	AY613797	*nad1*
Lemiox rimosus (Rafinesque, 1831)	UA20702	*HM230417	*nad1*
Lemiox rimosus	UA20702	*HM230406	*cox1*
Obovaria (*Pseudoon*) *olivaria* (Rafinesque, 1820)	UAM2636	*HM230420	*nad1*

Appendix 1. (Continued)

Taxon	Source	Accession	Gene
Obovaria (Pseudoon) olivaria	UAM2635-7	*HM230408	*cox1*
Obovaria jacksoniana (Frierson, 1912)	Campbell *et al.* 2005	AY655109	*nad1*
Obovaria unicolor (Lea, 1845)	UAM1262	*HM230407	*cox1*
Plectomerus dombeyanus (Valenciennes, 1827)	Campbell *et al.* 2005	AY655110	*nad1*
Plectomerus dombeyanus	Campbell *et al.* 2005	AY655011	*cox1*
Plethobasus cooperianus (Lea, 1834)	UAM3529	*HM230351	ITS1
Plethobasus cyphyus (Rafinesque, 1820)	Campbell *et al.* 2005	AY613799	*nad1*
Plethobasus cyphyus	Campbell *et al.* 2005	AY613828	*cox1*
Pleurobema (Sintoxia) cordatum (Rafinesque, 1820)	Morrison *et al.* in prep	EF619917	*cox1*
Pleurobema (Sintoxia) cordatum	Campbell *et al.* 2005	AY613804	*nad1*
Pleurobema (Sintoxia) cordatum Ff10	Burdick and White 2007	DQ298533	*cox1*
Pleurobema (Sintoxia) sintoxia (Rafinesque, 1820)	Graf and Ó Foighil 2000	AF156509	*cox1*
Pleurobema (Sintoxia) sintoxia	Campbell *et al.* 2005	AY613815	*nad1*
Pleurobema clava (Lamarck, 1819)	Campbell *et al.* 2005	AY613802	*nad1*
Pleurobema clava	Bogan and Hoeh 2000	AF231754	*cox1*
Pleurobema decisum (Lea, 1831)	Campbell *et al.* 2005	AY655112	*nad1*
Pleurobema decisum	Campbell *et al.* 2005	AY613832	*cox1*
Pleurobema pyriforme (Lea, 1857)	Campbell *et al.* 2005	AY613812	*nad1*
Pleurobema pyriforme	Campbell *et al.* 2005	AY613839	*cox1*
"Pleurobema" collina (Conrad, 1837)	Campbell *et al.* 2005	AY613803	*nad1*
"Pleurobema" collina	Campbell *et al.* 2005	AY613830	*cox1*
"Pleurobema" stabile (Lea, 1861)	Campbell *et al.* 2008	AY613816	*nad1*
"Pleurobema" stabile	Campbell *et al.* 2008	AY613842	*cox1*
Pleuronaia barnesiana (Lea, 1838)	Campbell *et al.* 2005	AY613791	*nad1*
Pleuronaia barnesiana	Campbell *et al.* 2005	AY613822	*cox1*
Pleuronaia barnesiana 2	UAM2007	*HM230418	*nad1*
Pleuronaia barnesiana 3	UAM3152	*HM230419	*nad1*
Pleuronaia dolabelloides 1 (Lea, 1840)	Campbell *et al.* 2005	AY655106	*nad1*
Pleuronaia dolabelloides 1	Campbell *et al.* 2005	AY655004	*cox1*
Pleuronaia dolabelloides 2	Campbell *et al.* 2005	AY613827	*cox1*
Pleuronaia dolabelloides 2	Campbell *et al.* 2005	AY613798	*nad1*
Pleuronaia gibbera (Lea, 1838)	Campbell *et al.* 2005	AY613808	*nad1*
Pleuronaia gibbera	Campbell *et al.* 2005	AY613835	*cox1*
Popenaias popeii (Lea, 1857)	Campbell *et al.* 2005	AY655118	*nad1*
Popenaias popeii	Campbell *et al.* 2005	AY655020	*cox1*
Quadrula quadrula (Rafinesque, 1820)	Serb *et al.* 2003	AY158772	*nad1*
Quadrula quadrula	Graf and Ó Foighil 2000	AF156511	*cox1*
Quadrula rumphiana (Lea, 1852)	UA20703	*HM230409	*cox1*
Quadrula rumphiana	UA20703	*HM230421	*nad1*
Reginaia ebenus (Lea, 1831)	UAM3149	*HM230352	ITS1
Reginaia ebenus	Campbell *et al.* 2005	AY655098	*nad1*
Reginaia ebenus 1	Campbell *et al.* 2005	AY654999	*cox1*
Reginaia ebenus 2	UAM3149	*HM230422	*nad1*
Reginaia ebenus 2	Burdick and White 2007	DQ298539	*cox1*
Reginaia rotulata (Wright, 1899)	Serb *et al.* 2003	AY158799	*nad1*
Reginaia rotulata 1	Lydeard *et al.* 2000	AF232813	*cox1*
Reginaia rotulata 2 (=1)	Lydeard *et al.* 2000	AF232814	*cox1*
Rotundaria infucata (Conrad, 1834)	Lydeard *et al.* 2000	AF232805	*cox1*
Rotundaria infucata 1	Campbell *et al.* 2005	AY655121	*nad1*
Rotundaria kleiniana (Lea, 1852)	Lydeard *et al.* 2000	AF232809	*cox1*
Rotundaria kleiniana 1	Serb *et al.* 2003	AY158795	*nad1*
Rotundaria refulgens (Lea, 1868)	Serb *et al.* 2003	AY158788	*nad1*

Appendix 1. (Continued)

Taxon	Source	Accession	Gene
Rotundaria refulgens	Chapman *et al.* 2008	EF033269	*cox1*
Rotundaria succissa 1 (Lea, 1852)	Serb *et al.* 2003	AY158792	*nad1*
Rotundaria succissa 1	Lydeard *et al.* 2000	AF232820	*cox1*
Rotundaria succissa 2	Lydeard *et al.* 2000	AF232819	*cox1*
Rotundaria succissa 2	Serb *et al.* 2003	AY158809	*nad1*
Rotundaria succissa A	Lydeard *et al.* 2000	AF232821	*cox1*
Rotundaria succissa B	Lydeard *et al.* 2000	AF232818	*cox1*
Rotundaria tuberculata (Rafinesque, 1820)	UAM1490	*HM230410	*cox1*
Rotundaria tuberculata	UAM1490	*HM230353	ITS1
Rotundaria tuberculata	Campbell *et al.* 2005	AY655088	*nad1*
Uniomerus declivis (Say, 1831)	Campbell *et al.* 2005	AY613846	*cox1*

Appendix 2. Collection data for new material.

Fusconaia "askewi" UAM3392, UAM3395: Drakes Creek, Calcasieu drainage, Vernon Parish Louisiana. Coll. S. Shively
Fusconaia cerina UAM3376: Twelvemile Creek at Rt. 1045, St. Helena Parish, Louisiana. Coll. S. Shively
Fusconaia cerina UAM3235: Little Silver Creek at Pleasant Hill Road, Washington Parish, Louisiana. Coll. S. Shively
Fusconaia cor UAM2605: Clinch River at Clinchport, Scott Co., Virginia. Coll. J. Fridell, M. Cantrell, S. Fraley
Fusconaia escambia UAM3403: Little Patsaliga Creek at CR35, 3.5 air miles SSW Rutledge, Crenshaw Co., Alabama. Coll. J. Williams, R. Lewis
Fusconaia flava UAM2648: Mississippi River, Marion Co., Missouri. Coll. B. Sietman
Fusconaia masoni NCSM-H: Nottoway River, Virginia.
Fusconaia ozarkensis UAM3500, UAM3501: Bull Creek, pool 1/4 mi E of Hwy 160, Taney Co., Missouri. Coll. M. C. Barnhart
Fusconaia subrotunda UA2266= UAM3237 (2, 4–6, 10–20, 22, 24–27, 29, 30, 32–35, 44): Hiwassee River, RM 100–101, downstream of new US64 bridge, Cherokee Co., North Carolina. Coll. J. A. Johnson, T. W. Savidge, J. M. Alderman
Fusconaia subrotunda UA 20698 (23, 28): Hiwassee River, near island above new US64 bridge, Cherokee Co., North Carolina. Coll. J. A. Johnson, T. W. Savidge, J. M. Alderman
Fusconaia subrotunda UA 20699 (45): Hiwassee River, upstream of island above new US64 bridge, Cherokee Co., North Carolina. Coll. J. A. Johnson, T. W. Savidge, J. M. Alderman
Fusconaia subrotunda UA 20700 (47): Hiwassee River, above Mission Dam, 100 mupstream of SR 1302 (Fires Creek Rd.) Bridge, Clay Co., North Carolina. Coll. J. A. Johnson, T. W. Savidge, J. M. Alderman
Fusconaia subrotunda UA 20701 (50): Hiwassee River, west side of sharp southward bend downstream of new US 64 bridge, Cherokee Co., North Carolina. Coll. J. A. Johnson, T. W. Savidge, J. M. Alderman
Lemiox rimosus UA 20702: Largest island at Lillard's Mill, side near dam, Duck River, Marshall Co., Tennessee. Coll. L. J. Levine
Obovaria unicolor UAM1262: Bogue Chitto River below sill, St. Tammany Parish, Louisiana Coll. P. Hartfield
Plethobasus cooperianus UAM3529: Tennessee River, River mile (RM) 170.2, Shallow Bluff Island, Decatur/Hardin Co., Tennessee. Coll. S. Ahlstedt
Pleuronaia barnesiana UAM2007: Nolichucky River at Bewley Island, Greene Co., Tennessee. Coll. S. Fraley, J. J. Jenkinson
Pleuronaia barnesiana UAM3152: Duck River, RM 178.8 below Lillard Mill dam, Marshall Co., Tennessee. Coll. S. Ahlstedt
Obovaria (Pseudoon) olivaria UAM2636: Mississippi River, Marion Co., Missouri Coll. B. Sietman
Quadrula rumphiana UA 20703: Sucarnoochee River above U.S. 11, Livingston, Sumter Co., Alabama Coll. D. Campbell, S. A. Clark
Reginaia ebenus UAM3149: Coosa River above Wetumpka, below Pipeline Shoals, Elmore Co., Alabama Coll. S. Ahlstedt
Rotundaria tuberculata UAM1490: Bear Creek downstream of Rock Creek confluence and Natchez Trace Pkwy, Colbert Co., Alabama Coll. K. J. Roe, J. M. Serb, S. McGreggor

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The genera of Pleurobemini (Bivalvia: Unionidae: Ambleminae)

David C. Campbell[1] and Charles Lydeard[2]

[1]Paleontological Research Institution, 1259 Trumansburg Road, Ithaca, New York 14850 U.S.A.

[2]The National Science Foundation, 4201 Wilson Boulevard, Arlington, Virginia 22230 U.S.A.

Correspondence, David C. Campbell: pleuronaia@gmail.com

Abstract: The unionid tribe Pleurobemini is diverse but poorly-understood phylogenetically. Current classification recognizes two highly diverse genera, *Elliptio* Rafinesque, 1819 and *Pleurobema* Rafinesque, 1820, besides the moderately diverse *Fusconaia* Simpson, 1900, and several genera with one to three species. However, classification at the species and genus level has been problematic. Molecular data and re-examination of shell morphology and anatomy indicate new groupings of these taxa. Several genera proposed by early workers such as Rafinesque and Swainson are available, but are poorly-characterized and are often overlooked. We analyzed two mitochondrial genes, *cox1* and *nad1*, for 50 species assigned to Pleurobemini, including the type species of each genus and as many other species as possible. Although the majority of studied species in *Elliptio*, *Pleurobema*, and *Fusconaia* show close affinities to the respective type species, the affinities of others are problematic. Genera or subgenera such as *Eurynia* Rafinesque, 1819, *Sintoxia* Rafinesque, 1820, and *Pleuronaia* Frierson, 1927, generally regarded as subjective synonyms, apply to some clades. Other clades or unaffiliated species have no available name. *Quincuncina burkei* (Walker, 1922), the type of the genus *Quincuncina* Ortmann, 1922, is assigned to *Fusconaia* Simpson, 1900. *Fusconaia apalachicola* (Williams and Fradkin, 1999), *F. ebenus* (Lea, 1831), *F. rotulata* (Wright, 1899) (also listed as *Obovaria rotulata*), *F. succissa* (Lea, 1852), *Cyclonaias tuberculatus* (Rafinesque, 1820), *Uniomerus* Conrad, 1853, and the *Quincuncina infucata* (Conrad, 1834) complex are all excluded from Pleurobemini. The first three are placed in the new genus *Reginaia* Campbell and Lydeard; *Uniomerus* Conrad, 1853 is assigned to Quadrulini; and the remainder belong in the *pustulosa* Lea, 1831 group of Quadrulini (genus *Rotundaria* Rafinesque, 1820).

Key words: systematics, nomenclature, freshwater mussels, Unioniformes

North America has the highest diversity of freshwater mussels (Unionoida) in the world, with > 300 species and > 50 genera. Current classification (Graf and Cummings 2007) assigns most of these taxa to the subfamily Ambleminae, which in turn is divided into the tribes Amblemini, Gonidini, Lampsilini, Pleurobemini, and Quadrulini. Recognition of Pleurobemini has developed gradually. Hannibal (1912) first recognized the Pleurobemini as a distinct group (as a subfamily), but the composition of the tribe varies among authors. Many workers allied or even synonymized it with the Old World tribe Unionini. Simpson (1914) also placed Margaritiferidae in this group. Most pre-cladistic morphology- and anatomy-based classifications of the Unionoida placed great weight on a single character such as gill modifications, breeding season, beak sculpture, or hinge, depending on the author. This neglected evidence for homoplasy, and also led to the use of symplesiomorphies as diagnostic features in classification. At the tribe level, *Fusconaia* Simpson, 1900 was grouped with *Quadrula* Rafinesque, 1820 rather than with Pleurobemini in the classifications of Simpson (1900, 1914) and Heard and Guckert (1971) due to its generally tetragenous brooding. Likewise, sculptured shells such as *Plethobasus* Simpson, 1900 and *Quincuncina* Ortmann, 1922 frequently were assigned to Quadrulini. Modell (1964) and others assigned the thin-shelled, weakly dentate *Hemistena* Rafinesque, 1820 to the alasmidontines (now considered a synonym of anodontines, Graf and Cummings 2007). Detailed anatomy (Ortmann 1911, Heard and Guckert 1971) showed that the Pleurobemini were not congeneric with Old World *Unio* Philipsson, 1788, but they often remained assigned to the same tribe or subfamily. Molecular analyses, starting with Davis and Fuller (1981), led to the recognition of Pleurobemini as a distinct member of the Ambleminae, only distantly related to *Unio*.

Within Pleurobemini, most species are assigned to *Elliptio* Rafinesque, 1820, *Pleurobema* Rafinesque, 1820, and *Fusconaia* Simpson, 1900, with *Elliptio* dominating the Atlantic drainage and *Pleurobema* and *Fusconaia* dominating the Gulf of Mexico drainages. Other genera sometimes assigned to Pleurobemini have one to four recognized species. Currently recognized genera include *Cyclonaias* Pilsbry, 1922, *Elliptoideus* Frierson, 1927, *Hemistena* Rafinesque, 1820, *Lexingtonia* Ortmann, 1914, *Plectomerus* Conrad, 1853, *Plethobasus*, *Popenaias* Frierson, 1927, *Quincuncina*, and *Uniomerus* Conrad, 1853. However, many of the poorly-characterized genera proposed by Rafinesque (1819, 1820, 1831, 1832) and Swainson (1840) have not been re-examined in light of modern data and usage.

Several species have been classified in different genera by different authors, especially the more poorly-known species from southern U. S. drainages. This reflects the general lack of obvious characters such as shell sculpture or specialized host-attraction structures that facilitate division of Quadrulini and Lampsilini into genera. Additionally, many of these species had not received much anatomical study. For example, *Pleurobema beadleianum* (Lea, 1861) has been placed in *Elliptio* and *Fusconaia* (Vidrine 1993), *Fusconaia rotulata* (Wright, 1899) has also been assigned to *Obovaria* (Williams *et al.* 2008), *Pleurobema collina* (Conrad, 1837) has been placed in *Alasmidonta*, *Canthyria*, *Lexingtonia*, and *Fusconaia* (Johnson 1970) and closely resembles *Elliptio steinstanana* Johnson and Clarke, 1983, and *Pleurobema riddellii* (Lea, 1862) has also been placed in *Fusconaia* (Vidrine 1993). Some authors make their genus assignments even more confusing by putting a formerly used genus in parentheses after the current genus, *e.g.* "*Villosa* (*Micromya*) *lienosa*". This format is standard for indicating a subgenus and should not be used for outdated genera; instead, a format such as "*Villosa lienosa* (formerly *Micromya*)" makes the intent clear (ICZN 1999, Recommendation 6A).

To provide new phylogenetic data, we used mitochondrial DNA sequencing. Genetic data provide an independent line of evidence for taxonomy, particularly useful when morphological and anatomical studies have had so much difficulty in confidently delimiting evolutionarily significant units. Although molecular data are not inherently superior to morphological data, they are of particular value when morphological variation is either slight or difficult to characterize, such as the subtle shape differences among many species in Pleurobemini. As Graf and Cummings (2006) point out, some published sequences are problematic, and different analyses of the same data can yield different results. However, additional sequences provide a check on previous work, and increasing both the number of taxa and the amount of data per taxon generally improves the robustness of phylogenetic results. Mitochondrial DNA can potentially follow a different evolutionary course from the main genetic heritage of a species, *e.g.*, through hybridization and introgression. But in freshwater mussels, the importance of distinct male and female mitochondrial lineages in the overall biology of the mussel makes discordant evolution of the mitochondria much less likely (Breton *et al.* 2011). A few species of Pleurobemini have been included in almost every previous molecular study on Ambleminae; several include multiple species (Lydeard *et al.* 2000, Kandl *et al.* 2001, Campbell *et al.* 2005, Burdick and White 2007, Campbell *et al.* 2008, and Campbell and Lydeard 2012). Representatives of other tribes were used as outgroups. We sequenced portions of two mitochondrial genes that had proven useful for species-level studies on unionids: cytochrome oxidase I (*cox1*) (Roe and Lydeard 1998) and NADH dehydrogenase I (*nad1*) (Buhay *et al.* 2002; Serb, Buhay *et al.* 2003). We also tried nuclear ITS1 sequences. Although it has worked for other unionids, ITS1 failed to give clear resolution within Pleurobemini (Campbell *et al.* 2008).

Our goal in this paper is to review the nomenclaturally available genera of Pleurobemini and to compare the nomenclature with the clades generated by analyses of molecular data for these taxa.

MATERIALS AND METHODS

We included sequences for 42 unambiguous species of Pleurobemini and 10 additional species that have been assigned to Pleurobemini by some authors, including the type species of all currently recognized genera and all available types of other genera. Outgroups were selected to represent the other tribes of Ambleminae. Table 1 lists the sequences generated in the present study, and Table 2 gives collection information for specimens with new data.

DNA extraction used either CTAB and chloroform-isoamyl alcohol protocols (Winnepenninckx *et al.* 1993) or Qiagen DNA extraction kits. The ITS1 region and portions of the *cox1* and *nad1* genes were amplified. Primers for *cox1* were 5′-GTTCCACAAATCATAAGGATATTGG-3′ and 5′-TACACCTCAGGGTGACCAAAAAACCA-3′, adapted from Folmer *et al.* (1994); primers for *nadh1* were 5′-TGGCA GAAAAGTGCATCAGATTTAAGC-3′ and 5′-GCTATTAGTA GGTCGTATCG-3′ (Buhay *et al.* 2002, Serb and Lydeard 2003); and primers for ITS1 were 5'-AAAAAGCTTCCG TAGGTGAACCTGCG-3' and 5'-AGCTTGCTGCGTTCTT CATCG-3' (King *et al.* 1999). For members of the Quadrulini, the primer LoGlyR (5'-CCTGCTTGGAAGGCAAGTGT ACT-3') (Serb, Buhay *et al.* 2003) often worked better as a reverse primer for *nadh1*. *Uniomerus* amplified with it but not with the above reverse primer (which also failed to amplify for Sommer (2007)). The forward primer UNIOCOII.2 from Walker *et al.* (2007) and/or the reverse primer HCOout (CCAGGTAAAATTAAAATATAAACTTC, Carpenter and Wheeler 1997) provided good amplification for *cox1* for some species. PCR cycles were: 92 °C 2 min; 92 °C 40 s, 40 °C 40 s, 72 °C 90 s 5×; 92 °C 40 s, 50 °C 40 s, 72 °C 90 s 25×; 72 °C 10 min; hold 4 °C. PCR products were purified using Qiagen QIAquick PCR purification kits and, if necessary, Qiagen gel extraction kits. Cycle sequencing used ABI Big Dye Terminator kits with thermal cycle parameters of 1 °C per second ramp speed, starting with 1 min at 96 °C followed by 26 cycles of 96 °C for 10 s, 49 °C for 5 s, and 60 °C for 4 min, then 10 min at 60 °C and hold at 4 °C. The cycle sequencing products were purified with Qiagen DyeEx kits and then run on an automated sequencer.

The results for each strand were compared and aligned using BioEdit (Hall 1999). We analyzed the sequences with TNT (Goloboff *et al.* 2008). Maximum parsimony analyses

Table 1. Sequences used. UAUC and UA are different versions of the University of Alabama catalog.

Taxon	Source	Accession	Gene
Amblema elliottii (Lea, 1856)	Campbell *et al.* 2005	AY654991	*cox1*
Amblema elliottii	Campbell *et al.* 2005	AY655086	*nad1*
Amblema plicata (Say, 1817)	Chapman, Piontkivska *et al.* 2008	EF033258	*cox1*
Amblema plicata	Serb, Buhay *et al.* 2003	AY158796	*nad1*
Dromus dromas (Lea, 1834)	Campbell *et al.* 2005	AY654993	*cox1*
Dromus dromas	UAUC3156	JF326439*	*nad1*
Elliptio arca	Campbell *et al.* 2005	AY654995	*cox1*
Elliptio arca	Campbell *et al.* 2005	AY655093	*nad1*
Elliptio arctata	Campbell *et al.* 2008	DQ383427	*cox1*
Elliptio arctata	UAUC3496	JF326440*	*nad1*
Elliptio complanata	Gangloff *et al.* in review	EU448172	*cox1*
Elliptio complanata	Kneeland and Rhymer 2007	EF446099	*nad1*
Elliptio crassidens	Campbell *et al.* 2008	DQ383428	*cox1*
Elliptio crassidens	Campbell *et al.* 2005	AY613788	*nad1*
Elliptio mcmichaeli	Gangloff *et al.* in review	EU377573	*cox1*
Elliptio mcmichaeli	UA3088	JF326441*	*nad1*
Elliptio pullata	Gangloff *et al.* in review	EU377571	*cox1*
Elliptio pullata	Gangloff *et al.* in review	EU380666	*nad1*
Elliptio purpurella	Gangloff *et al.* in review	EU377574	*cox1*
Elliptio purpurella	UA3569	JF326442*	*nad1*
Elliptio shepardiana	Gangloff *et al.* in review	EU448252	*nad1*
Elliptio (*Eurynia*) *dilatata*	Graf and Ó Foighil 2000	AF156507	*cox1*
Elliptio dilatata	Campbell *et al.* 2005	AY613789	*nad1*
Elliptoideus sloatianus	Campbell *et al.* 2005	AY613821	*cox1*
Elliptoideus sloatianus	Campbell *et al.* 2005	AY613790	*nad1*
Epioblasma rangiana (Lea, 1838)	new-clip from Virginia Tech	JF326432*	*cox1*
Epioblasma rangiana	Zanatta and Murphy 2006	DQ220720	*nad1*
Fusconaia burkei	Lydeard *et al.* 2000	AF232804	*cox1*
Fusconaia burkei	Serb, Buhay *et al.* 2003	AY158793	*nad1*
Fusconaia cerina	Campbell *et al.* 2005	AY613823	*cox1*
Fusconaia cerina	Campbell *et al.* 2005	AY613792	*nad1*
Fusconaia cor	Campbell *et al.* 2005	AY654997	*cox1*
Fusconaia cor	Campbell *et al.* 2005	AY655096	*nad1*
Fusconaia cuneolus	Campbell *et al.* 2005	AY654998	*cox1*
Fusconaia cuneolus	Campbell *et al.* 2005	AY655097	*nad1*
Fusconaia escambia	Lydeard *et al.* 2000	AF232816	*cox1*
Fusconaia escambia	Campbell and Lydeard 2011	HM230413*	*nad1*
Fusconaia flava	Bogan and Hoeh 2000	AF231733	*cox1*
Fusconaia flava	Campbell *et al.* 2005	AY613793	*nad1*
Fusconaia masoni	Campbell and Lydeard 2011	HM230371*	*cox1*
Fusconaia masoni	Campbell and Lydeard 2011	HM230415*	*nad1*
Fusconaia ozarkensis	Campbell and Lydeard 2011	HM230373*	*cox1*
Fusconaia ozarkensis	Campbell and Lydeard 2011	HM230416*	*nad1*
Fusconaia subrotunda	Campbell *et al.* 2005	AY613824	*cox1*
Fusconaia subrotunda	Campbell *et al.* 2005	AY613794	*nad1*
Glebula rotundata (Lamarck, 1819)	Chapman, Piontkivska *et al.* 2008	EF033264	*cox1*
Glebula rotundata	Campbell *et al.* 2005	AY613795	*nad1*
Hemistena lata	Campbell *et al.* 2005	AY613825	*cox1*
Hemistena lata	Campbell *et al.* 2005	AY613796	*nad1*
Lampsilis ovata (Say, 1817)	Chapman, Piontkivska *et al.* 2008	EF033262	*cox1*
Lampsilis ovata	Campbell *et al.* 2005	AY613797	*nad1*
Lampsilis virescens (Lea, 1858)	new clip from Alabama Aquatic Biodiversity Center	JF326433*	*cox1*

Table 1. (Continued)

Taxon	Source	Accession	Gene
Lampsilis virescens	new clip from Alabama Aquatic Biodiversity Center	JF326443*	*nad1*
Lemiox rimosus (Rafinesque, 1831)	Campbell and Lydeard 2011	HM230406*	*cox1*
Lemiox rimosus	Campbell and Lydeard 2011	HM230417*	*nad1*
Obliquaria reflexa (Rafinesque, 1820)	Chapman, Piontkivska *et al.* 2008	EF033254	*cox1*
Obliquaria reflexa	Campbell *et al.* 2005	AY655008	*nad1*
Obovaria jacksoniana	Campbell *et al.* 2005	AY655009	*cox1*
Obovaria jacksoniana	Campbell *et al.* 2005	AY655109	*nad1*
Obovaria (Pseudoon) olivaria	Chapman, Piontkivska *et al.* 2008	EF033267	*cox1*
Obovaria (Pseudoon) olivaria	Campbell and Lydeard 2011	HM230420*	*nad1*
Plectomerus dombeyanus (Valenciennes, 1827)	Chapman, Piontkivska *et al.* 2008	EF033252	*cox1*
Plectomerus dombeyanus	Campbell *et al.* 2005	AY655110	*nad1*
Plethobasus cyphyus	Campbell *et al.* 2005	AY613828	*cox1*
Plethobasus cyphyus	Campbell *et al.* 2005	AY613799	*nad1*
Pleurobema athearni	Campbell *et al.* 2005	AY655015	*cox1*
Pleurobema athearni	Campbell *et al.* 2005	AY655114	*nad1*
Pleurobema beadleianum	Campbell *et al.* 2008	DQ383429	*cox1*
Pleurobema beadleianum	Campbell *et al.* 2008	AY613800	*nad1*
Pleurobema clava	Campbell *et al.* 2005	AY655013	*cox1*
Pleurobema clava	Campbell *et al.* 2005	AY613802	*nad1*
Pleurobema collina	Campbell *et al.* 2005	AY613830	*cox1*
Pleurobema collina	Campbell *et al.* 2005	AY613803	*nad1*
Pleurobema decisum	Campbell *et al.* 2005	AY613832	*cox1*
Pleurobema decisum	Campbell *et al.* 2005	AY655112	*nad1*
Pleurobema furvum	Campbell *et al.* 2005	AY613833	*cox1*
Pleurobema furvum	Campbell *et al.* 2005	AY613806	*nad1*
Pleurobema georgianum	Campbell *et al.* 2005	AY613834	*cox1*
Pleurobema georgianum	Campbell *et al.* 2005	AY613807	*nad1*
Pleurobema hanleyianum	Campbell *et al.* 2005	AY613836	*cox1*
Pleurobema hanleyianum	Campbell *et al.* 2005	AY655115	*nad1*
Pleurobema oviforme	Campbell *et al.* 2005	AY655017	*cox1*
Pleurobema oviforme	Campbell *et al.* 2005	AY613810	*nad1*
Pleurobema perovatum	new Wilson Creek	*JN180999	*cox1*
Pleurobema perovatum	Campbell *et al.* 2005	AY613811	*nad1*
Pleurobema pyriforme	Campbell *et al.* 2005	AY613839	*cox1*
Pleurobema pyriforme	Campbell *et al.* 2005	AY613812	*nad1*
Pleurobema stabile	Campbell *et al.* 2008	AY613842	*cox1*
Pleurobema stabile	Campbell *et al.* 2008	AY613816	*nad1*
Pleurobema strodeanum	Campbell *et al.* 2005	AY613843	*cox1*
Pleurobema strodeanum	Campbell *et al.* 2005	AY613817	*nad1*
Pleurobema taitianum	Campbell *et al.* 2005	AY613844	*cox1*
Pleurobema taitianum	Campbell *et al.* 2005	AY613818	*nad1*
Pleurobema (Sintoxia) cordatum	Campbell *et al.* 2005	AY613831	*cox1*
Pleurobema (Sintoxia) cordatum	Campbell *et al.* 2005	AY613804	*nad1*
Pleurobema (Sintoxia) riddellii	specimen 186	JF326434*	*cox1*
Pleurobema (Sintoxia) riddellii	specimen 186	JF326444*	*nad1*
Pleurobema (Sintoxia) rubrum	Campbell *et al.* 2005	AY613841	*cox1*
Pleurobema (Sintoxia) rubrum	UAUC529	JF326445*	*nad1*
Pleurobema (Sintoxia) sintoxia	Graf and Ó Foighil 2000	AF156509	*cox1*
Pleurobema (Sintoxia) sintoxia	Campbell *et al.* 2005	AY613815	*nad1*
Pleuronaia barnesiana	Campbell *et al.* 2005	AY613822	*cox1*
Pleuronaia barnesiana	Campbell and Lydeard 2011	HM230418*	*nad1*
Pleuronaia dolabelloides	Campbell *et al.* 2005	AY613827	*cox1*

Table 1. (Continued)

Taxon	Source	Accession	Gene
Pleuronaia dolabelloides	Campbell *et al.* 2005	AY613798	*nad1*
Pleuronaia gibbera	Campbell *et al.* 2005	AY613808	*nad1*
Pleuronaia gibbera	Campbell *et al.* 2008	DQ383432	*cox1*
Popenaias popeii	Campbell *et al.* 2005	AY655020	*cox1*
Popenaias popeii	Campbell *et al.* 2005	AY655118	*nad1*
Potamilus alatus	Bogan and Hoeh 2000	AF231752	*cox1*
Potamilus alatus	Campbell *et al.* 2005	AY655119	*nad1*
Potamilus capax (Green, 1832)	Roe and Lydeard 1998	AF049513	*cox1*
Potamilus capax	UAUC36	JF326446*	*nad1*
Quadrula nobilis	Lydeard *et al.* 2000	AF232823	*cox1*
Quadrula nobilis	new specimen, Sucarnochee River	JF326447*	*nad1*
Quadrula quadrula	Chapman, Piontkivska *et al.* 2008	EF033268	*cox1*
Quadrula quadrula	Breton *et al.* 2009	FJ809750	*nad1*
Quadrula rumphiana (Lea, 1852)	Campbell and Lydeard 2011	HM230409*	*cox1*
Quadrula rumphiana	Campbell and Lydeard 2011	HM230421*	*nad1*
Reginaia ebenus	Campbell *et al.* 2005	AY654999	*cox1*
Reginaia ebenus	Campbell and Lydeard 2011	HM230422*	*nad1*
Reginaia rotulata	Lydeard *et al.* 2000	AF232813	*cox1*
Reginaia rotulata	Serb, Buhay *et al.* 2003	AY158799	*nad1*
Rotundaria infucata	Lydeard *et al.* 2000	AF232806	*cox1*
Rotundaria infucata	Campbell *et al.* 2005	AY655121	*nad1*
Rotundaria kleiniana	Lydeard *et al.* 2000	AF232809	*cox1*
Rotundaria kleiniana	Serb, Buhay *et al.* 2003	AY158795	*nad1*
Rotundaria pustulosa	Henley *et al.* 2006	DQ640237	*nad1*
Rotundaria refulgens (Lea, 1868)	Chapman, Piontkivska *et al.* 2008	EF033269	*cox1*
Rotundaria refulgens	Serb, Buhay *et al.* 2003	AY158788	*nad1*
Rotundaria succissa	Lydeard *et al.* 2000	AF232820	*cox1*
Rotundaria succissa	Serb, Buhay *et al.* 2003	AY158792	*nad1*
Rotundaria tuberculata	Campbell and Lydeard 2011	HM230410*	*cox1*
Rotundaria tuberculata	Campbell *et al.* 2005	AY655088	*nad1*
Theliderma metanevra	new specimen, Cahaba River	JF326435*	*cox1*
Theliderma metanevra	new specimen, Cahaba River	JF326448*	*nad1*
Toxolasma lividum (Rafinesque, 1831)	new specimen, Tennessee River	JF326436*	*cox1*
Toxolasma lividum	new specimen, Tennessee River	JF326449*	*nad1*
Tritogonia verrucosa (Rafinesque, 1820)	Graf and Cummings 2006	DQ191413	*cox1*
Tritogonia verrucosa	Serb, Buhay *et al.* 2003	AY158791	*nad1*
Uniomerus declivis	Campbell *et al.* 2005	AY613846	*cox1*
Uniomerus declivis	UAUC3290	JF326450*	*nad1*
Uniomerus tetralasmus	new specimen, Hale County, Alabama	JF326437*	*cox1*
Uniomerus tetralasmus	new specimen, Hale County, Alabama	JF326451*	*nad1*
Venustaconcha ellipsiformis (Conrad, 1836)	Chapman, Piontkivska *et al.* 2008	EF033260	*cox1*
Venustaconcha ellipsiformis	Zanatta and Murphy 2006	DQ220722	*nad1*
Venustaconcha ellipsiformis 2	Campbell *et al.* 2005	AY655026	*cox1*
Venustaconcha ellipsiformis 2	Campbell *et al.* 2005	AY655126	*nad1*
Venustaconcha pleasii (Marsh, 1891)	UAUC3520, 3525(identical)	JF326438*	*cox1*
Venustaconcha pleasii	UAUC3520	JF326452*	*nad1*
Villosa fabalis (Lea, 1831)	Zanatta and Murphy 2006	DQ220726	*cox1*
Villosa fabalis	Zanatta and Murphy 2006	DQ220723	*nad1*

* indicates new sequences.

Table 2. Collection data for new material

Taxon	Collection #	Locality	Collector
Dromus dromas	UAUC3156	36.5756°N, 83.3664°W Powell River at McDowell Shoal, Hancock County, Tennessee	unknown
Elliptio arctata	UAUC3496	33.1853°N, 87.0002°W, Cahaba River at Booth's Ford, Shelby County, Alabama	P. Johnson
Elliptio mcmichaeli	UAUC3088	30.7773°N, 85.8497°W, Choctawhatchee River at U.S. Highway 90, Holmes County, Florida	Blalock, Hill, and Lewis
Elliptio purpurella	UAUC3569	31.0847°N, 85.1942°W, Rocky Creek at CR8, Houston County, Alabama	Campbell, Clark, Chesser
Epioblasma rangiana	clip from Virginia Tech	Allegheny River at Hunter Station Bridge, Venango County, Pennsylvania	Virginia Tech
Lampsilis virescens	clip from Alabama Aquatic Biodiversity Center	Estill Fork, Paint Rock River, Jackson County, Alabama	P. Johnson
Potamilus capax	UAUC36	35.5872°N, 90.4397°W, Iron Mines Creek, 1.25 mi W of Highway 140, Poinsett County, Arkansas	J. L. Harris, R. Doster
Quadrula nobilis	new	Sucarnoochee River above U.S. 11, Livingston, Sumter County, Alabama	D. Campbell, S. A. Clark
Pleurobema (*Sintoxia*) *riddellii*	186	Village Creek, Hardin County, Texas	L. Burlakova, Alex Karatayev
Pleurobema (*Sintoxia*) *rubra*	UAUC529	35.8825°N, 91.2186°W, Strawberry River 1 mi below Simstown Spring, off State Route 42, Sharp County, Arkansas	K. J. Roe *et al.*
Theliderma metanevra	new specimen	Cahaba River at Barton Beach, Perry County, Alabama	Thornhill
Toxolasma lividum	new specimen	Tennessee River mile 249, Colbert/Lauderdale County, Alabama	J. T. Garner, A. C. Suddith
Uniomerus declivis	UAUC3290	Big Sunflower River at Farrell Road Bridge, ~5 mi N of Clarksdale, Coahoma County, Mississippi	W. R. Haag, J. L. Stanton
Uniomerus tetralasmus	new specimen	32.6737°N, 87.7647°W, small creek at County Route 24, Hale County, Alabama	D. Campbell, T. Campbell
Venustaconcha pleasii	UAUC3520	Beaver Creek T25N R17W S12, Douglas County, Missouri	M. C. Barnhart
Venustaconcha pleasii	UAUC3525	Beaver Creek T25N R17W S12, Douglas County, Missouri	M. C. Barnhart

used 500 random replicates, using all the "new technology" methods (ratchet, drifting, sectorial searches, and fusing). Jackknife analyses used 500 replicates, each using a random parsimony search of 10 replicates. MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) was run for the whole data set with 2,000,000 generations, 8 chains, and sample frequency of 100; revmat, shape, pinvar, and statefreq were unlinked, and burnin was set to 5000. To test the strength of support for dividing *Pleurobema* and *Elliptio*, analyses were run including only Pleurobemini. These had 400,000 generations, 8 chains, and sample frequency of 100. Burnin was set to 1000. All of the analyses had *Elliptio sensu stricto* (*i.e.*, excluding *Eurynia* Rafinesque, 1819), *Eurynia*, *Pleurobema sensu stricto* (*i.e.*, excluding "*Pleurobema*" *collina*, "*Pleurobema*" *stabile*, *Pleuronaia gibbera*, and *Sintoxia*), and *Sintoxia* constrained individually as monophyletic. One of the analyses also specified an *Elliptio-Eurynia* clade, and another specified a *Pleurobema-Sintoxia* clade. Twice the difference in the harmonic means of the ln likelihood posterior probabilities for two different models approximates the value for the Bayes factor table of Kass and Raftery (1995) (Nylander *et al.* 2004).

RESULTS

ITS1 sequences failed to provide resolution within Pleurobemini, but they did support the exclusion of *F. ebenus* (Lea, 1831) from Pleurobemini. Although Pleurobemini as a whole and a few species-level subclades within Pleurobemini were recognizable with ITS1, most taxa formed an unresolved polytomy. In contrast, the mitochondrial genes provided good resolution.

Several clades were well-supported in the present analyses (Figs. 1–3). As in previous molecular analyses (Campbell *et al.*

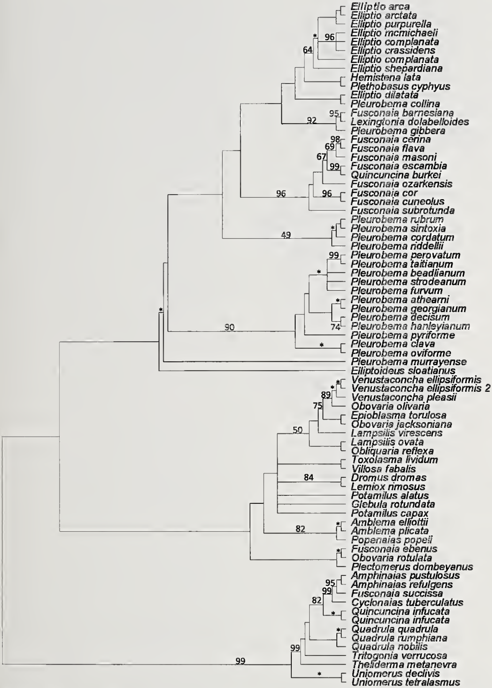
Elliptio arca
Elliptio arctata
Elliptio purpurella
Elliptio mcmichaeli
Elliptio complanata
Elliptio crassidens
Elliptio complanata
Elliptio shepardiana
Hemistena lata
Plethobasus cyphyus
Elliptio dilatata
Pleurobema collina
Fusconaia barnesiana
Lexingtonia dolabelloides
Pleurobema gibbera
Fusconaia cerina
Fusconaia flava
Fusconaia masoni
Fusconaia escambia
Quincuncina burkei
Fusconaia ozarkensis
Fusconaia cor
Fusconaia cuneolus
Fusconaia subrotunda
Pleurobema rubrum
Pleurobema sintoxia
Pleurobema cordatum
Pleurobema riddellii
Pleurobema perovatum
Pleurobema taitianum
Pleurobema beadlianum
Pleurobema strodeanum
Pleurobema furvum
Pleurobema athearni
Pleurobema georgianum
Pleurobema decisum
Pleurobema hanleyianum
Pleurobema pyriforme
Pleurobema clava
Pleurobema oviforme
Pleurobema murrayense
Elliptoideus sloatianus
Venustaconcha ellipsiformis
Venustaconcha ellipsiformis 2
Venustaconcha pleasii
Obovaria olivaria
Epioblasma torulosa
Obovaria jacksoniana
Lampsilis virescens
Lampsilis ovata
Obliquaria reflexa
Toxolasma lividum
Villosa fabalis
Dromus dromas
Lemiox rimosus
Potamilus alatus
Glebula rotundata
Potamilus capax
Amblema elliottii
Amblema plicata
Popenaias popeii
Fusconaia ebenus
Obovaria rotulata
Plectomerus dombeyanus
Amphinaias pustulosus
Amphinaias refulgens
Fusconaia succissa
Cyclonaias tuberculatus
Quincuncina infucata
Quincuncina infucata
Quadrula quadrula
Quadrula rumphiana
Quadrula nobilis
Tritogonia verrucosa
Theliderma metanevra
Uniomerus declivis
Uniomerus tetralasmus
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Figure 1. Strict consensus maximum parsimony tree (12 trees, length = 4647), *cox1* and *nad1* data combined. Taxa are labeled by the genera in Graf and Cummings (2007). These are the same as in Turgeon *et al.* (1998) except that *metanevra, pustulosa,* and *refulgens* were all listed as *Quadrula* in Turgeon *et al.* (1998) and both overlooked *Q. nobilis* (Conrad, 1854), recognized in Howells *et al.* (1996). Numbers on branches are jackknife percentages. * = 100% support.

2005, Campbell *et al.* 2008), Pleurobemini (*Pleurobema, Elliptio, Eurynia, Fusconaia, Hemistena, Plethobasus, Pleuronaia* Frierson, 1927, *Sintoxia* Rafinesque, 1820) was well-supported, with *Elliptoideus* Frierson, 1927 as a possible basal sister taxon. Relationships between Pleurobemini genera remain poorly-resolved. Support for most genera, including *Fusconaia,* is strong once apparently misclassified species are excluded. "*Fusconaia*" *ebenus* and "*F.*" *rotulata* are closely related to each other, but not strongly associated with any other sampled species. "*Fusconaia*" *barnesiana* (Lea, 1838) is closely related to "*Lexingtonia*" *dollabelloides* (Lea, 1840) and "*Pleurobema*" *gibberum* (Lea, 1838). "*Pleurobema*" *cordatum* (Rafinesque, 1820), "*P.*" *rubrum* (Rafinesque, 1820), and "*P.*" *sintoxia* (Rafinesque, 1820) form a clade, and "*P.*" *riddellii* is moderately supported as a member of this clade. "*Elliptio*" *dilatata* (Rafinesque, 1820), "*Pleurobema*" *stabile* (Lea, 1861), and "*Pleurobema*" *collina* place within Pleurobemini but do not strongly group with any other species.

Although the Bayesian consensus tree did not contradict a sister-taxon relationship between *Eurynia* and *Elliptio sensu stricto* and weakly supported a sister-taxon relationship between *Pleurobema sensu stricto* and *Sintoxia*, calculation of Bayes factors supports the unconstrained tree over specifying monophly of *Pleurobema* plus *Sintoxia* or *Elliptio* plus *Eurynia*. Kass and Raftery (1995) identify such values as moderately to strongly supporting the unconstrained tree; nevertheless, the Bayes factors are small compared to the differences made by changing evolutionary models (Nylander *et al.* 2004). This suggests that the criteria for "strong" support might need to be modified for phylogenetic analyses.

DISCUSSION

The presence of a few well-supported clades in a background of generally poor resolution for ITS1 suggests that it has undergone occasional purifying selection but more often has freely diverged, possibly affected by ancestral polymorphisms or hybridization. The ITS1 sequence for *Rotundaria tuberculata* (Rafinesque, 1820) includes a partial duplication of the gene region (a similar but longer partial duplication was also observed in *Strophitus undulatus* (Say, 1816) [Campbell, pers. obs.]; this would explain the

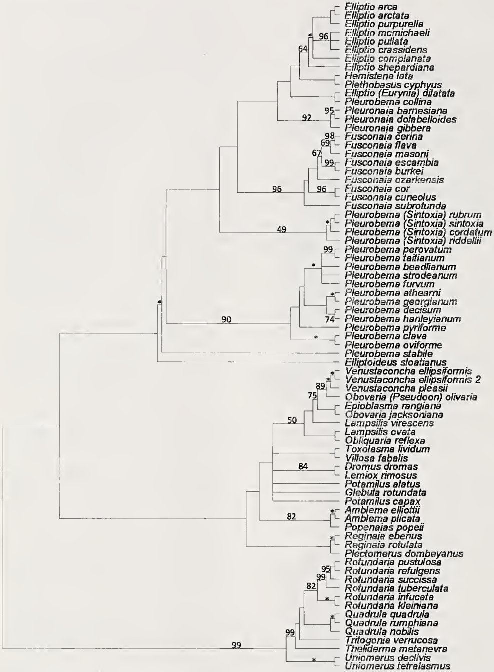
Elliptio arca
Elliptio arctata
Elliptio purpurella
Elliptio mcmichaeli
Elliptio pullata
Elliptio crassidens
Elliptio complanata
Elliptio shepardiana
Hemistena lata
Plethobasus cyphyus
Elliptio (Eurynia) dilatata
Pleurobema collina
Pleuronaia barnesiana
Pleuronaia dolabelloides
Pleuronaia gibbera
Fusconaia cerina
Fusconaia flava
Fusconaia masoni
Fusconaia escambia
Fusconaia burkei
Fusconaia ozarkensis
Fusconaia cor
Fusconaia cuneolus
Fusconaia subrotunda
Pleurobema (Sintoxia) rubrum
Pleurobema (Sintoxia) sintoxia
Pleurobema (Sintoxia) cordatum
Pleurobema (Sintoxia) riddellii
Pleurobema perovatum
Pleurobema taitianum
Pleurobema beadlianum
Pleurobema strodeanum
Pleurobema furvum
Pleurobema athearni
Pleurobema georgianum
Pleurobema decisum
Pleurobema hanleyianum
Pleurobema pyriforme
Pleurobema clava
Pleurobema oviforme
Pleurobema stabile
Elliptoideus sloatianus
Venustaconcha ellipsiformis
Venustaconcha ellipsiformis 2
Venustaconcha pleasii
Obovaria (Pseudoon) olivaria
Epioblasma rangiana
Obovaria jacksoniana
Lampsilis virescens
Lampsilis ovata
Obliquaria reflexa
Toxolasma lividum
Villosa fabalis
Dromus dromas
Lemiox rimosus
Potamilus alatus
Glebula rotundata
Potamilus capax
Amblema elliottii
Amblema plicata
Popenaias popeii
Reginaia ebenus
Reginaia rotulata
Plectomerus dombeyanus
Rotundaria pustulosa
Rotundaria refulgens
Rotundaria succissa
Rotundaria tuberculata
Rotundaria infucata
Rotundaria kleiniana
Quadrula quadrula
Quadrula rumphiana
Quadrula nobilis
Tritogonia verrucosa
Theliderma metanevra
Uniomerus declivis
Uniomerus tetralasmus
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Figure 2. Strict consensus maximum parsimony tree (12 trees, length = 4647), *cox1* and *nad1* data combined. Numbers on branches are jackknife percentages. * = 100% support. Genera revised to reflect the results of the present analyses.

anomalously long fragment in White *et al.* (1996)). As the duplicate regions are not identical, independent reduction of such an ancestral polymorphism to normal length could produce a mosaic of features in the descendants.

The present data support previous molecular studies that indicate a need for revision of the genus-level classification of Pleurobemini (see also systematic taxonomy section, below) (Lydeard *et al.* 2000, Serb, Buhay *et al.* 2003, Campbell *et al.* 2005, Campbell *et al.* 2008). A few species placed in Pleurobemini belong in other tribes. "*Fusconaia*" *succissa* (Lea, 1852) belongs within Quadrulini, as do "*Quincuncina*" *infucata* (Conrad, 1834), "*Quincuncina*" *kleiniana* Lea, 1852, and "*Cyclonaias*" *tuberculatus* (Rafinesque, 1820), a result also found in part by Lydeard *et al.* (2000), Serb, Buhay *et al.* (2003) [the sequence for *C. tuberculatus* in that paper was incorrect; it was from a tissue clip received along with one for *Potamilus alatus* (Say, 1817) and evidently a mixup occurred before or after arrival at the lab], Campbell *et al.* (2005), and Williams *et al.* (2008). All four species are members of the *pustulosa* group of species in Quadrulini. Several genus- or subgenus-level names are available for this group, but the oldest turns out to be *Rotundaria* Rafinesque, 1820. Additionally, the molecular data ally *Uniomerus* with Quadrulini rather than with Pleurobemini (present study).

"*Fusconaia*" *ebenus* and "*Obovaria*" or "*Fusconaia*" *rotulata* form a distinctive clade (Lydeard *et al.* 2000, Campbell *et al.* 2005), probably also including the extinct "*Fusconaia*" *apalachicola*. Williams and Fradkin, 1999. The relationships of this clade to the rest of Ambleminae are not well-resolved. Several authors proposed that these species were closely related on morphological and anatomical grounds (Stansbery 1971, Williams and Butler 1994, Athearn 1998, Williams and Fradkin 1999, Williams *et al.* 2008). As no genus was available for this group, we propose the new genus *Reginaia* Campbell and Lydeard.

Several other taxa belong within Pleurobemini, but are not supported as members of their assigned genus. "*Pleurobema*" *collina* and "*Pleurobema*" *stabile* are clearly within Pleurobemini but not consistently associated with any other sampled taxa. The remaining species currently assigned to *Pleurobema* and *Fusconaia* form four main clades within Pleurobemini. "*Fusconaia*" *barnesiana*, "*Lexingtonia*" *dollabelloides*, and "*Pleurobema*" *gibberum* form one clade (Campbell *et al.* 2005). The genus *Pleuronaia* is available for this group (Williams *et al.* 2008). "*Pleurobema*"

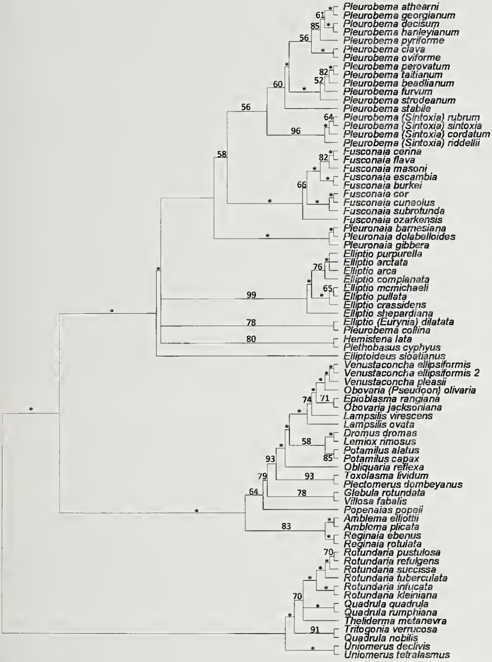
Pleurobema athearni
Pleurobema georgianum
Pleurobema decisum
Pleurobema hanleyianum
Pleurobema pyriforme
Pleurobema clava
Pleurobema oviforme
Pleurobema perovatum
Pleurobema taitianum
Pleurobema beadlianum
Pleurobema furvum
Pleurobema strodeanum
Pleurobema stabile
Pleurobema (Sintoxia) rubrum
Pleurobema (Sintoxia) sintoxia
Pleurobema (Sintoxia) cordatum
Pleurobema (Sintoxia) riddellii
Fusconaia cerina
Fusconaia flava
Fusconaia masoni
Fusconaia escambia
Fusconaia burkei
Fusconaia cor
Fusconaia cuneolus
Fusconaia subrotunda
Fusconaia ozarkensis
Pleuronaia barnesiana
Pleuronaia dolabelloides
Pleuronaia gibbera
Elliptio purpurella
Elliptio arctata
Elliptio arca
Elliptio complanata
Elliptio mcmichaeli
Elliptio pullata
Elliptio crassidens
Elliptio shepardiana
Elliptio (Eurynia) dilatata
Pleurobema collina
Hemistena lata
Plethobasus cyphyus
Elliptoideus sloatianus
Venustaconcha ellipsiformis
Venustaconcha ellipsiformis 2
Venustaconcha pleasii
Obovaria (Pseudoon) olivaria
Epioblasma rangiana
Obovaria jacksoniana
Lampsilis virescens
Lampsilis ovata
Dromus dromas
Lemiox rimosus
Potamilus alatus
Potamilus capax
Obliquaria reflexa
Toxolasma lividum
Plectomerus dombeyanus
Glebula rotundata
Villosa fabalis
Popenaias popeii
Amblema elliottii
Amblema plicata
Reginaia ebenus
Reginaia rotulata
Rotundaria pustulosa
Rotundaria refulgens
Rotundaria succissa
Rotundaria tuberculata
Rotundaria infucata
Rotundaria kleiniana
Quadrula quadrula
Quadrula rumphiana
Theliderma metanevra
Tritogonia verrucosa
Quadrula nobilis
Uniomerus declivis
Uniomerus tetralasmus

Figure 3. Bayesian consensus tree. Numbers on branches are posterior probabilities. * = 100% support. Genera revised to reflect the results of the present analyses. Final average standard deviation of the split frequencies was 0.009192, most likely tree had a ln likelihood of -21057.05, with burnin set to 5000 the mean ln likelihood was -21063.02.

cordatum, "*Pleurobema*" *sintoxia*, and "*Pleurobema*" *rubrum* form a second small clade; probable relatives include "*Pleurobema*" *riddellii* (Lea, 1862) and "*Pleurobema*" *plenum* (Lea, 1840) (Morrison *et al.* 2005, present study). This group has long been recognized on morphological and anatomical grounds (Ortmann 1918, Frierson 1927, Stansbery 1967, Watters *et al.* 2009). The genus *Sintoxia* is available for this group of species. This clade is sometimes but not consistently resolved as sister to *Pleurobema* and so might be treated as a subgenus of *Pleurobema*. The remaining species of *Pleurobema* form a large clade, including the type species of *Pleurobema*, and the remaining species of *Fusconaia* form a large clade, including the type species of *Fusconaia*, *Quincuncina*, and *Lexingtonia* (Williams *et al.* 2008). *Elliptio* also appears polyphyletic, with *E. dilatata* failing to group with other species of that genus; unpublished data suggest that a few additional species of *Elliptio* may also belong elsewhere (Serb, Bogan *et al.* 2003, McCartney and Wilbur 2007, Sommer 2007). However, the support for separating *Eurynia* and *Sintoxia* from *Elliptio* and *Pleurobema*, respectively, is relatively low, so for the present we treat these as subgenera to draw attention to their distinctiveness.

Systematic Taxonomy

Genera that have been assigned to or confused with Pleurobemini are listed below with tribe assignments.

Family Unionidae
Subfamily Ambleminae

Tribe Pleurobemini Hannibal, 1912 (= Elliptionini Modell, 1942)

Several genus-level names have been proposed for taxa in this tribe or affiliated groups. Many are from the work of Rafinesque and of Swainson, who unfortunately did not designate type species in most cases (except by monotypy or tautonomy) and often had poor descriptions and figures. Thus, determining type species requires surveying subsequent literature until the first subsequent type designation is found. Genera from Mexico, Central America, and Eurasia have also sometimes been assigned to Pleurobemini (Frierson 1927, Modell 1964, Starobogatov 1970, Nagel and Badino 2001). The relationships of Mexican and Central American unionids are poorly-known, often with no anatomical information, but biogeographically it is plausible that some might belong to Pleurobemini. As we have no new information about them, they are not listed here. No Eurasian taxa seem to be validly placed in any tribe of Ambleminae from eastern North America (Campbell *et al.* 2005, Altaba and Altaba 2009).

Genus ***Canthyria*** Swainson, 1840

Type species: *Unio spinosus* Lea, 1836 by original designation.

Considered a subjective synonym of *Elliptio* (Serb, Bogan *et al.* 2003)

Genus ***Cunicula*** Swainson, 1840

Type species: *Unio purpurascens* Lamarck, 1819 by subsequent designation (Herrmannsen 1846), a subjective synonym of *Elliptio complanata* (Lightfoot, 1786).

Considered a subjective synonym of *Elliptio* (Gangloff *et al.* in review, present analysis).

Genus ***Curricula*** Gray, 1847

Error for *Cunicula* Swainson, 1840. Gray's type designation of *Unio planulatus* Lea, 1829 would make this a subjective senior synonym of *Ptychobranchus* Simpson, 1900, but fortunately Herrmannsen's type designation of *Unio purpurascens* for the correctly spelled genus is older.

Genus ***Elliptio*** Rafinesque, 1819

Type species: *Unio* (*Elliptio*) *nigra* Rafinesque, 1820 (a subjective synonym of *Elliptio crassidens* (Lamarck, 1819)) by subsequent designation (Ortmann 1912). Simpson (1900) listed *crassidens* as the type, not a valid designation as it was not an originally included species.

Other included taxa in this study: *E. arca* (Conrad, 1834), *E. arctata* (Conrad, 1834), *Elliptio complanata* (Lightfoot, 1786), *Elliptio mcmichaeli* Clench and Turner, 1956, *Elliptio pullata* (Lea, 1856), *Elliptio purpurella* (Lea, 1857), and *Elliptio shepardiana* (Lea, 1834).

Taxa excluded from *Elliptio sensu stricto*: *Elliptio* (*Eurynia*) *dilatata* (Rafinesque, 1820)

Genus ***Elliptoideus*** Frierson, 1927

Type species: *Unio sloatianus* Lea, 1840, by original designation.

Other included taxa: none

The relationships of this genus to Pleurobemini are not very strongly supported. Further study is desirable.

Genus ***Ensinaia*** Starobogatov, 1970

Type species: *Unio sheppardianus* [sic] Lea, 1834, error for *Elliptio shepardiana* (Lea, 1834), by original designation.

Considered a subjective synonym of *Elliptio* (Gangloff *et al.* in review, Morrison *et al.* 2005, present results).

Genus ***Eurynia*** Rafinesque, 1819

Type species: *Unio dilatata* Rafinesque, 1820, by subsequent designation in Herrmannsen (1847).

Other included taxa: none

Agassiz (1852) overlooked Herrmannsen's type designations for several taxa and used *Eurynia* for the group now called *Ligumia*. His usage was followed by several other authors. More confusion comes from the many misspellings: Vokes (1980) lists *Eurinea*, *Euryma*, *Eurynaia*, *Eurynea*, and *Rurynea*. Although *Elliptio* (*Eurynia*) *dilatata* has generally been placed in *Elliptio* on morphological and anatomical grounds (Ortmann and Walker 1922a), molecular studies consistently fail to group it with *Elliptio* and weakly associate it with other taxa instead (Campbell *et al.* 2005, Campbell *et al.* 2008, McCartney and Wilbur 2007, Gangloff *et al.* in review).

The shell is more elongate than most species of *Elliptio sensu stricto*, but some Atlantic drainage species are also elongate and might be related to *Elliptio* (*Eurynia*) *dilatata* rather than to *Elliptio crassidens*. *Elliptio arca*, from the Mobile basin, is very similar conchologically to *Elliptio* (*Eurynia*) *dilatata*, but molecular data place *Elliptio arca* with *Elliptio crassidens*.

Genus ***Fusconaia*** Simpson, 1900

Type species: *Unio trigonus* Lea, 1831 (a subjective synonym of *Fusconaia flava* (Rafinesque, 1820)) by original designation.

Other included taxa in this study: *Fusconaia burkei* (Walker, 1922), *Fusconaia cerina* (Conrad, 1838), *Fusconaia cor* (Conrad, 1834), *Fusconaia cuneolus* (I. Lea, 1840), *Fusconaia escambia* Clench and Turner, 1956, *Fusconaia masoni* (Conrad, 1834), *Fusconaia ozarkensis* (Call, 1887), and *Fusconaia subrotunda* (I. Lea, 1831).

Taxa excluded from *Fusconaia*: *Reginaia ebenus* (Lea, 1831), *Reginaia rotulata* (B. H. Wright, 1899), *Reginaia apalachicola* (Williams and Fradkin, 1999), *Rotundaria succinea* (Lea, 1852), and *Pleuronaia barnesiana* (Lea, 1838).

Genus ***Hemilastena*** Agassiz, 1852

Invalid emendation of *Hemistena* Rafinesque, 1820.

Genus ***Hemistena*** Rafinesque, 1820

Type species: *Anodonta* (*Lastena*) *lata* Rafinesque, 1820 by monotypy.

Other included taxa: none

Genus ***Lexingtonia*** Ortmann, 1914

Type species: *Unio subplanus* Conrad, 1837 by original designation.

Considered a subjective synonym of *Fusconaia*. Williams *et al.* (2008) report that molecular data indicate that *L. subplana* is a synonym of *F. masoni*, and present data indicate that *F. masoni* belongs in *Fusconaia*.

Genus *Lintoxia* "Rafinesque, 1820"

Error for *Sintoxia* Rafinesque, 1820. Cited in Vokes (1980), as not seen; presumably a typographical error in one of his sources. Now listed in several nomenclatural compilations. Also purportedly altered to *Lyntoxia* by Agassiz (1846), an error for *Syntoxia*, an invalid emendation actually present in Agassiz.

Genus *Luteacarnea* Frierson, 1927–1929?

Type species: *Unio cooperianus* Lea, 1834, designated herein. *Luteacarnea* is a replacement name for *Striata* Frierson, 1927. The originally designated type species was *Obovaria striata* Rafinesque, 1820, which Frierson (1927) regarded as a senior synonym of *Unio cooperianus*, a usage followed by some other authors. However, Parmalee and Bogan (1998), Williams *et al.* (2008), and Watters *et al.* (2009) identified *O. striata* as a synonym of *Obovaria subrotunda* Rafinesque, 1820. Given the uncertainty about the identity of *Obovaria striata*, we believe it would best promote stability in nomenclature to unambiguously associate the genus with the species Frierson had in mind. Therefore, we designate *U. cooperianus* as the type species of *Luteacarnea* under article 70.3 of the ICZN Code (ICZN 1999).

Although Haas (1969b) and Modell (1964) place this genus in Quadrulini, current classification treats it as a subjective synonym of *Plethobasus*. We obtained an ITS1 sequence that supports its inclusion in Pleurobemini but does not provide clear evidence of its affinities within the tribe; attempted amplification of other genes has not been successful.

This name was proposed as a replacement name for *Striata* Frierson, 1927 in the corrigenda to Frierson (1927). The absence of the corrigenda in many copies and its apparently arbitrary placement either at the front or the back of the book indicate that it was not a part of the original publication. Unfortunately, there are no indications in the corrigenda of when it was published, and the publisher's records were lost in a flood years ago (Carey C. Newman, pers. comm.). Because it seems to have been issued separately from the main text, the corrigenda seems to qualify as a separate publication, but its date of publication is unclear. The first published reference to names in the corrigenda that we have located is Strecker (1929), who lists the names from the corrigenda as if they were a part of the original and does not explicitly reference the corrigenda as a separate item (the cited pagination in Strecker (1929) does not mention it). This suggests that the corrigendum was published fairly closely with the original, as does the fact that the typeface matches (one copy seen had a typewritten copy of the corrigenda, presumably added by a later owner). This and the other genera from the corrigenda to Frierson (1927) have sometimes been credited to Thiele, 1934, presumably by authors who lacked access to the corrigenda and so were unable to find the genus in Frierson's work.

Genus *Obliquata* Frierson, 1927

Type species: *Obliquaria* (*Scalenaria*) *obliquata* Rafinesque, 1820 (a subjective synonym of *Sintoxia rubra* Rafinesque, 1820), by original designation.

Considered a subjective synonym of *Sintoxia* Rafinesque, 1820.

Genus *Odatelia* Rafinesque, 1832

Type species: *Odatelia radiata* Rafinesque, 1832, by monotypy (other species listed as synonyms); a subjective synonym of *Hemistena lata* (Rafinesque, 1820).

Considered a subjective synonym of *Hemistena*.

Genus *Plethobasus* Simpson, 1900

Type species: *Unio aesopus* Green, 1827 (a subjective synonym of *Plethobasus cyphus* (Rafinesque, 1820))

Other included taxa: *Plethobasus cicatricosus* (Say, 1829), *Plethobasus cooperianus* (Lea, 1834). Both of these species are highly imperiled and no definitive molecular data are available; however, the strong morphological similarities make their placement in *Plethobasus* very likely.

Genus *Pleurobema* Rafinesque, 1819

Type species: *Unio mytiloides* Rafinesque, 1820 by subsequent designation (Herrmannsen 1847) (a subjective synonym of *Pleurobema clava* (Lamarck, 1819))

Other included taxa in this study:

Pleurobema athearni Gangloff *et al.*, 2006, *Pleurobema beadlianum* (Lea, 1861), *Pleurobema decisum* (Lea, 1831), *Pleurobema furvum* (Conrad, 1834), *Pleurobema georgianum* (Lea, 1841), *Pleurobema hanleyianum* (Lea, 1852), *Pleurobema oviforme* (Conrad, 1834), *Pleurobema perovatum* (Conrad, 1834), *Pleurobema pyriforme* (Lea, 1857), *Pleurobema strodeanum* (Wright, 1898), and *Pleurobema taitianum* (Lea, 1834).

Taxa excluded from *Pleurobema sensu stricto*: "*Pleurobema*" *collina* (Conrad, 1837), *P.* (*Sintoxia*) *cordatum* (Rafinesque, 1820), *Pleuronaia gibbera* (Lea, 1838), *P.* (*Sintoxia*) *plenum* (Lea, 1840), *P.* (*Sintoxia*) *riddellii* (Lea, 1862), *P.* (*Sintoxia*) *rubrum* (Rafinesque, 1820), and *P.* (*Sintoxia*) *sintoxia* (Rafinesque, 1820). "*Pleurobema*" *stabile* (Lea, 1861) is not consistently supported as a member of the genus, but not consistently excluded, either.

Genus ***Pleuronaia*** Frierson, 1927

Type species: *Unio barnesianus* Lea, 1838 by original designation

Other included taxa: *Pleuronaia gibbera* (Lea, 1838), *Pleuronaia dolabelloides* (Lea, 1840) (Williams *et al.* 2008).

Nomenclatural note: Frierson also misspelled this name as *Pleuronia*. We select *Pleuronaia* as having priority over *Pleuronia* because the spelling *Pleuronia* occurs only once in Frierson (1927), because the *-naia* ending is common in unionids, and because *Pleuronaia* is the spelling used by subsequent authors.

Genus ***Quincuncina*** Ortmann, 1922 in Ortmann and Walker (1922b)

Type species: *Quincuncina burkei* Walker, 1922, by original designation.

Ortmann and Walker (1922b) established the genus because of the distinctive sculpture of the type species. However, previous (Lydeard *et al.* 2000, Serb, Buhay *et al.* 2003, Campbell *et al.* 2005) and present molecular data, in addition to the close anatomical similarity (Williams *et al.* 2008), indicate that *burkei* belongs in *Fusconaia*. "*Quincuncina*" *infucata* (Conrad, 1834) and "*Quincuncina*" *kleiniana* (Lea, 1852) belong in the *pustulosa* group of *Quadrula* (Serb, Buhay *et al.* 2003, Williams *et al.* 2008, present data). The affinities of "*Quincuncina*" *mitchelli* (Simpson, 1895) are unknown; it may be extinct (Burlakova *et al.* 2011).

This genus is considered a junior subjective synonym of *Fusconaia* (Williams *et al.* 2008).

Genus ***Sayunio*** de Gregorio, 1914

Type species: *Unio dehiscens oriensopsis* de Gregorio, 1914 (a subjective synonym of *Hemistena lata* (Rafinesque, 1820)) by original designation.

Considered a subjective synonym of *Hemistena*.

Genus ***Scalenaria*** Rafinesque, 1820

Type species: *Obliquaria* (*Scalenaria*) *scalenia* Rafinesque, 1820 (a subjective synonym of *Pleurobema clava* (Lamarck, 1819)), by subsequent designation (Herrmannsen 1848)

Considered a subjective synonym of *Pleurobema*.

Genus ***Sintoxia*** Rafinesque, 1820

Type species: *Obliquaria sintoxia* Rafinesque, 1820, by tautonomy.

Other included taxa: *Sintoxia cordata* (Rafinesque, 1820), *Sintoxia plena* (Lea, 1840), *Sintoxia riddellii* (Lea, 1862), *Sintoxia rubra* (Rafinesque, 1820). The *cordatum* group has been widely recognized (*e.g.*, Ortmann 1918, Frierson 1927, Stansbery 1967), but there is much uncertainty about the number of species. Frierson (1927) assigned *Sintoxia riddellii* to this group, but most other studies have focused on the Mississippi drainage taxa. It may not be monophyletic (Burdick and White 2007, Morrison *et al.* 2005). Some analyses place it close to *Pleurobema*, so we currently treat it as a subgenus rather than a genus. *Syntoxia* is an unjustified emendation.

Genus ***Striata*** Frierson, 1927

Type species: *Unio cooperianus* Lea, 1834, designated herein. The originally designated type species was *Obovaria striata* Rafinesque, 1820, but the identity of Rafinesque's species is debated. This genus name is a junior homonym and was replaced with *Luteacarnea* Frierson, 1927–1929? See further discussion under *Luteacarnea*. We designate *Unio cooperianus* as the type species of *Striata* under article 70.3 of the ICZN Code. *Striata* Frierson is a junior subjective synonym of *Plethobasus*.

Tribe uncertain

(This includes poorly-characterized names as well as well-studied taxa that are not clearly affiliated with a named tribe)

Genus ***Aximedia*** Rafinesque, 1820

Type species: Rafinesque (1820) included three new species: *Unio* (*Aximedia*) *elliptica* Rafinesque, 1820; *U.* (*A.*) *levigata* Rafinesque (1820) (he also suggested it might instead belong in *Obliquaria* (*Plagiola*) Rafinesque, 1820); and *U.* (*A.*) *zonalis* Rafinesque, 1820. No type species has been selected in the literature that we have examined. *U. elliptica* is considered a synonym of *Pleurobema clava* by Parmalee and Bogan (1998); *U. levigata* is considered a synonym of *Obovaria subrotunda* by Parmalee and Bogan (1998) and *U. zonalis* is considered a synonym of *Obovaria subrotunda* by Haas (1969a). Since *Pleurobema* applies to *P. clava*, whereas the precise relationship of *Obovaria subrotunda* to *O. retusa*, the type of *Obovaria*, is unknown, it might be more useful to have *Aximedia* apply to *levigata* or *zonalis*. We leave formal type designation for someone doing a revision of the *Obovaria-Epioblasma-Venustaconcha* clade.

Genus ***Bariosta*** Rafinesque, 1831

Type species: *Unio ponderosus* Rafinesque, 1831, by subsequent designation (Frierson 1914). Williams *et al.* (2008) cite Fischer (1886) as designating a type;

however, Fischer sometimes identified the cited species in his descriptions as the type and sometimes just as an example. In his list of unionid generic names, he does not specify whether the species cited are types or examples, and taking them to be types would disrupt existing usage for *Toxolasma* and possibly other genera. Although Frierson (1914) thought that *Unio ponderosus* was recognizable as *Plectomerus dombeyanus* (Valenciennes, 1827; Frierson cited *Unio trapezoides* Lea, 1831, a subjective synonym of *dombeyanus*), other authors have not agreed. Williams *et al.* (2008) declared *Unio ponderosus* and *Bariosta* to be *nomina dubia* and *Bariosta* to be a *nomen oblitum.*

Genus ***Flexiplis*** Rafinesque, 1831

Type species: *Anodonta digonata* Rafinesque, 1831, by monotypy.

Haas (1969b) synonymized *Flexiplis* with *Hemistena*, but the identity of *A. digonata* is highly doubtful (even differing in Haas 1969a). Williams *et al.* (2008) declared *Flexiplis* and *Anodonta digonata* to be *nomina dubia* and *Flexiplis* to be a *nomen oblitum.*

Genus ***Popenaias*** Frierson, 1927

Type species: *Unio popeii* Lea, 1857 by original designation

Other included taxa: Additional species in Mexico and Central America have been assigned to *Popenaias* (Graf and Cummings 2007), but they have not been available for molecular studies. *Elliptio buckleyi* (Lea, 1843) was placed with *Popenaias* by Heard and Guckert (1971) based on the breeding season, but all other data place it with *Elliptio*, including molecular data (Gangloff *et al.* in review). Chapman, Gordon *et al.* (2008) found that *Popenaias* is closely related to Lampsilini.

Genus ***Reginaia*** Campbell and Lydeard, new genus

Type species: *Unio ebenus* Lea, 1831

Other included species: *Reginaia rotulata* (Wright, 1899), *Reginaia apalachicola* (Williams and Fradkin, 1999). *Reginaia apalachicola* is assigned to this genus on morphological grounds; it is known only from prehistoric middens.

Diagnosis: Shell relatively oval, unsculptured; beaks strong and anterior; posterior slope rounded, with a weaker posterior ridge than in *Fusconaia* (Fig. 4). Periostracum dark, especially in mature individuals, without rays, unlike most *Fusconaia*. Very distinct based on molecular data (Lydeard *et al.* 2000, Serb, Buhay *et al.* 2003, Campbell *et al.* 2005, Burdick and White 2007, Campbell and Lydeard 2012, present data), not clearly assignable to any recognized tribe. The shell is rounded posteroventrally, whereas typical *Fusconaia* has a well-developed posterior ridge producing an angle at the posteroventral end (less well-developed in *F. subrotunda*). Beaks are less anterior in most true *Fusconaia* than in *Reginaia* (*F. cor* and *F. subrotunda* may have similarly anterior beaks). Umbonal cavity in *Reginaia* deep and compressed; in *Fusconaia* beak cavities less compressed. *Fusconaia* often has a small accessory anterior

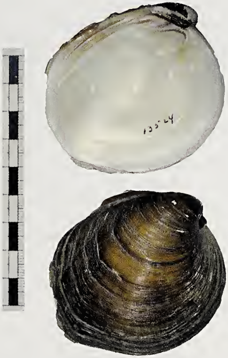

Figure 4. *Reginaia ebenus* (Lea, 1831), the type species of *Reginaia* n. gen. (University of Rochester 13524 from "Ohio", now in Paleontological Research Institution collections). Scale bar in cm.

Table 3. Bayes factors. "Constraint" is what group was constrained as monophyletic. "Mean ln likelihood" is the harmonic mean of the likelihood values (excluding burnin). The Bayes factor table uses twice the difference between the likelihoods for the models being compared (in this case, between the unconstrained and constrained trees). In this case, it indicates the degree to which the unconstrained model is favored over the one with the constraint. A value of 6 to 10 is strong support, over 10 is very strong support (Kass and Raftery 1995, Nylander *et al.* 2004).

Constraint	Mean ln likelihood	2*ln (Bayes factor)
Unconstrained	-9574.26	not applicable
Elliptio + Eurynia	-9576.56	4.6
Pleurobema + Sintoxia	-9577.60	6.68

pseudocardinal which we have not observed in *Reginaia*. The conglutinate tends to fragment, unlike most *Fusconaia* (Barnhart *et al*. 2008). Differs from Lampsilini and Pleurobemini except for *Fusconaia* in being tetragenous. The *Pleurobema* (*Sintoxia*) group can resemble *Reginaia* in shell form, but they have a flattened area or sulcus in front of the posterior ridge and are ectogenous.

Etymology: Conflation of *regina*, a queen, and *naias*, a nymph, commonly used as a designation for the pearly freshwater mussels. "*Regina*" reflects the common name of ebonyshell for *ebenus* and pigtoe for *Fusconaia* and refers to the Empress of Blandings, a fictional prize black pig (Wodehouse 1927).

Genus ***Uniopsis*** Swainson, 1840

Type species: *Uniopsis mytiloides* Swainson, 1840 by monotypy. Identity uncertain.

Swainson initially identified the species as *Uniopsis radiata* in part I of his work. Swainson's footnote suggests that his specimen might be "*Margaritana radiata* of Lea's Synopsis", but Swainson admitted that this identification was a guess based only on the species name. This is a rather cryptic reference to *Alasmodonta radiata* Conrad, 1834 (now *Anodontoides radiatus*). In part II, Swainson renames it *Uniopsis mytiloïdes* [the umlaut is now omitted in accord with the modern ICZN code (ICZN 1999)]. Presumably the change of species name in part II of Swainson reflected a decision that it wasn't *radiata* of Lea's list. Swainson confused things further by mentioning a conspecific (or possibly congeneric; he calls it "of this type") specimen that had been labeled "*Alasmodon inflata*". We have not located any plausible source for this name and assume it was a manuscript name; published unionoid species named "*inflata*" or "*inflatus*" before 1840 are clearly not similar.

Swainson's figure is somewhat similar to elongate *Pleurobema* species such as *Pleurobema clava* (a senior synonym of *Unio mytiloides* Rafinesque, 1820), but Swainson said it had no lateral teeth. The color pattern of fine radial lines in his figure (if accurate) is also atypical for *Pleurobema*. The lack of pseudolaterals and Swainson's assignment of the genus to "Alasmodontinae" [sic] have generally suggested some species of *Alasmidonta* or a related genus to most authors.

Simpson (1900) identified *Uniopsis mytiloides* as a synonym of *Alasmidonta undulata* Say, 1816, and most authors have followed this identification. However, Modell (1964) listed the genus as a synonym of *Pleurobema*. This probably is based on identification of *Uniopsis mytiloides* as a new genus assignment for *Unio mytiloides* Rafinesque, 1820 rather than as a new species of Swainson's. That interpretation is very unlikely, because Swainson assigned *Unio mytiloides* Rafinesque to *Unio sensu stricto* both in part I and part II of his work (he used *Unio* for *Pleurobema* and placed true *Unio* in another genus). Instead, it seems most likely that Swainson named his new species after Rafinesque's, because he thought that *Uniopsis* was an alasmidontine analogue of "*Unio*". Watters *et al*. (2009) question Simpson's identification and instead suggest that Swainson had a non-North American specimen. Thus, the identity of *Uniopsis* is unsettled, but it is unlikely to belong in Pleurobemini.

Tribe Quadrulini Ihering, 1901

Genus ***Cyclonaias*** Pilsbry in Ortmann and Walker, 1922a

Type species: *Obliquaria* (*Rotundaria*) *tuberculata* Rafinesque, 1820, by original designation.

Some authors have placed this genus in Pleurobemini based on its usually ectobranchous brooding; however, it otherwise closely resembles other Quadrulini. This is an objective synonym of *Rotundaria*.

Genus ***Rotundaria*** Rafinesque, 1820

Type species: *Obliquaria* (*Rotundaria*) *tuberculata* Rafinesque, 1820, by subsequent designation (Valenciennes 1827).

Following Agassiz (1852), many workers used *Rotundaria* for *Cyclonaias tuberculatus* (*e.g.*, Simpson 1900, 1914, Ortmann 1920, Modell 1949). Ortmann and Walker (1922a) pointed out that Herrmannsen (1848) had designated *Obliquaria* (*Rotundaria*) *subrotunda* Rafinesque, 1820 as the type of *Rotundaria*, although they ignored other type designations in Herrmannsen (1846–1852). Therefore, they created *Cyclonaias* Pilsbry in Ortmann and Walker, 1922a as a replacement name for *Rotundaria sensu* Agassiz. But there is an older type designation for *Rotundaria*, in Valenciennes (1827), who stated that Rafinesque had sent a lot of *tuberculatus* to the museum in Paris as the type of the new genus *Rotundaria* ("comme le type d'un nouveau genre qu'il nomme *Rotundaria*", p. 231). This also makes the well-known "*Cyclonaias*" *tuberculatus* the type of this genus, whereas the next-oldest genus name for the "*Quadrula*" *pustulosa* (Lea, 1831) group, *Amphinaias*, is based on the poorly-known and probably extinct *Unio couchianus* Lea, 1860 from the Rio Grande system.

The "*Quadrula pustulosa*" group has been widely recognized on conchological grounds (Simpson 1914, Frierson 1927, Serb, Buhay *et al*. 2003, Graf and Cummings 2007, Watters *et al*. 2009). It includes species with a relatively round shell, in contrast to the squarer shells of the *Q. quadrula* (Rafinesque, 1820) group and the strong posterior ridge of the "*Q*". *metanevra* (Rafinesque, 1820) group or the elongate shape of *Tritogonia* Agassiz, 1852. Most individuals have nodes, as typical for quadrulines, but they do not have the large, projecting knobs of many other quadrulines. The only reasons for separating "*Cyclonaias*" *tuberculatus* from the *pustulosa* group are the usually ectobranchous brooding and the coppery-purple nacre (Ortmann and Walker 1922a). Although the pattern of gill use in brooding is an important feature in unionoid classification, no one feature (including DNA) is immune to convergence, and the report of partial tetrabranchy in *tuberculatus* (Frierson, 1927) suggests that not much weight should be placed on ectobranchy in this case. Previous molecular data has supported recognition of the *pustulosa* group (Serb, Buhay *et al.* 2003). The host-attraction and glochidial release patterns vary somewhat between the groups of Quadrulini (Barnhart *et al.* 2008), but better documentation for more species is needed to confirm the generality of these patterns.

We follow Graf and Cummings (2007) in treating this group as a separate genus, although it could also be treated as a subgenus of a broadly defined *Quadrula*. Watters *et al.* (2009) also suggest recognizing the *pustulosa* group as a distinct genus but do not identify a suitable genus name.

Subsequent subjective or objective synonyms include *Amphinaias* Fischer and Crosse, 1894 (type species, *Unio couchianus* Lea, 1860 by original designation), which is the genus used in Graf and Cummings 2007; *Cyclonaias* Pilsbry, 1922 (type species, *Obliquaria (Rotundaria) tuberculata* Rafinesque, 1820 by original designation); *Bullata* Frierson, 1927 (type species, *Unio pustulosus* Lea, 1831 by original designation; invalid--a junior homonym of the marginellid gastropod genus *Bullata* Jousseaume, 1875); and *Pustulosa* Frierson, 1927–1929? (type species, *Unio pustulosus* Lea, 1831 by original designation).

Rotundaria tuberculata often uses only the outer gills for brooding, which has led some authors to place it in Pleurobemini (Graf and Cummings 2007); however, shell morphology, other anatomy (Williams *et al.* 2008), and molecular data (present results) place it in Quadrulini.

Genus *Theliderma* Swainson, 1840

Type species: *Obliquaria* (*Quadrula*) *metanevra* Rafinesque, 1820 by subsequent designation (as *metanera* [*sic*]), Gray (1847) (other misspellings include *metanerva*, *metaneurus*, *metanever*, and *metaniver*). This is a subjective senior synonym of *Orthonymus* Agassiz, 1852 (type species, *Unio cylindricus* Say, 1817, original designation). Simpson (1900, 1914) treated *metanevra* as the type of *Quadrula* and used *Theliderma* for *Quadrula sensu stricto*, but his type designation for *Quadrula* is invalid (the type of *Quadrula* is *Obliquaria* (*Quadrula*) *quadrula* Rafinesque, 1820 by tautonomy) and for *Theliderma* Simpson's designation is well subsequent to Gray. *Theliderma* was used by Graf and Cummings (2007), and *Orthonymus* was treated as a valid genus for the *metanevra* group by authors such as Modell (1964) and Davis and Fuller (1981). The present discussion is mainly intended to explain the discrepancy with Simpson's usage. Molecular data (Serb, Buhay *et al.* 2003) as well as morphological data (Simpson 1914, Graf and Cummings 2007) support this as a distinct group.

Genus *Uniomerus* Conrad, 1853

Type species: *Unio declivis* Say, 1831 (subsequent designation, Frierson 1927, 1927–1929?). Earlier designations of *Unio tetralasmus* (Say, 1831) as the type fall afoul of the fact that Conrad failed to include that species in the original description, though he did include some subjective synonyms of it. Frierson (1927–1929?) indicated that the species listed on p. 9–11 of Frierson (1927) were the types of the respective genera, thus making his mention of *U. declivis* a type designation.

Other included taxa: *Uniomerus carolinensis* (Bosc, 1801), *Uniomerus columbensis* (Lea, 1857), *Uniomerus tetralasmus* (Say, 1831).

Although morphological and anatomical studies have generally placed *Uniomerus* in Pleurobemini, molecular data place it in Quadrulini. Some species of *Uniomerus* are easily confused with *Elliptio*, but *Uniomerus* almost always can be recognized by the two parallel grooves on the posterior side of the posterior ridge.

Tribe Lampsilini Ihering, 1901

Genus *Lastena* Rafinesque, 1820

Type species: *Anodonta* (*Lastena*) *ohiensis* Rafinesque, 1820, by subsequent designation (Herrmannsen 1847), now placed in *Potamilus* Rafinesque, 1818.

This genus was occasionally used for *Hemistena lata* (based on a later type designation) or *Utterbackia imbecilis* (Say, 1829) (based on misidentification of *ohiensis*) by earlier authors. Considered a subjective synonym of *Potamilus*.

Genus *Lostena* Gray, 1847

Error for *Lastena* Rafinesque, 1820.

Genus *Obovaria* Rafinesque, 1819

Type species: *Unio retusa* Lamarck, 1819, by ICZN Opinion 2188 (ICZN 2008).

Prevalent usage reflected the incorrect assumption that *Obovaria retusa* Rafinesque, 1819 was the same as *Unio retusa* Lamarck, 1819, but in fact Rafinesque was unaware of Lamarck's work at the time (Bogan *et al.* 2006). The type designation of *Obovaria* in Herrmannsen (1847) made *Obovaria obovalis* Rafinesque, 1820 the type of the genus. Agassiz (1852) selected *O. torsa* Rafinesque, 1820, a subjective synonym of *Unio retusa* Lamarck, as type, a designation followed by several authors (Simpson 1900, Ortmann and

Walker 1922a [though aware of Herrmannsen's designation], Modell 1964). *Obovaria obovalis* is widely recognized as a neglected senior synonym of *Unio ebenus* Lea, 1831 (Say 1834, Williams *et al.* 2008). However, current usage of *Obovaria* and *ebenus* are so well established that there seems little point in re-establishing Rafinesque's names in this group. The ICZN (2008) designated *Unio retusa* Lamarck, 1819 as the type of *Obovaria* and made *Obovaria obovalis* a *nomen oblitum* (Opinion 2188 2008). This follows traditional usage, but *Obovaria retusa* is on the brink of extinction and no molecular data are yet available, making it somewhat inconvenient as the type of the genus. Anatomically, *O. retusa* is similar to other species currently assigned to *Obovaria* (Ortmann 1912, Williams *et al.* 2008). Molecular data (Campbell *et al.* 2005, Zanatta and Murphy 2006, present results) indicate that "*Obovaria*" *olivaria* (Rafinesque, 1820) is relatively distinct from other *Obovaria* and may be closer to *Venustaconcha*. This suggests that greater weight should be given to the anatomical and conchological differences that prompted Simpson (1900) to propose *Pseudoon* for *olivaria*, and suggests that there may be unappreciated variation within *Obovaria*. (Note: the sequences identified in Campbell *et al.* (2005) as *Venustaconcha pleasii* Marsh, 1891 are in fact *V. ellipsiformis* (Lea, 1838); the *cox1* sequence AY655025 from Campbell *et al.* (2005) labeled as *Venustaconcha ellipsiformis* is a *Ptychobranchus*). Watters *et al.* (2009) refer to *O. subrotunda* as the type of *Obovaria* in their etymology, perhaps a lapse for its being the species Herrmannsen designated as type of *Rotundaria*. They identify *retusa* as the type under "Type species".

Genus ***Pseudoon*** Simpson, 1900

Type species: *Unio ellipsis* Lea, 1828 (a subjective synonym of *Obovaria olivaria* (Rafinesque, 1820)) by original designation. Simpson spelled it *Pseudoön*, but diacritical marks are not allowed under current ICZN rules (ICZN 1999).

Ortmann (1912) claimed that *Obovaria castanea* (Lea, 1831), a primary homonym now known as *O. jacksoniana* (Frierson, 1912), was intermediate between *Pseudoon* and standard *Obovaria* in anatomy, but for taxa sampled so far, *O. jacksoniana* seems to closely match other *Obovaria* in DNA (Campbell *et al.* 2005). *Pseudoon* is confusingly similar in spelling to *Pseudodon* Gould, 1844, an Asian unionid genus. Further study of the *Epioblasma-Obovaria-Venustaconcha* clade may support its recognition as a full genus; we use it as a subgenus here to draw attention to the apparent difference between *olivaria* and the rest of *Obovaria*. *Pseudoon* is neuter, so the species would become *Pseudoon olivarium*.

Genus ***Stenelasma*** Herrmannsen, 1849.

Invalid emendation of *Lastena* Rafinesque, 1820.

SUMMARY

Past and present molecular data suggest that several groups of unionid species do not match currently recognized genera. Several genus names in the literature, currently treated as subjective synonyms, are available, but their identity is often obscure or unclear. A review of available genera in Pleurobemini and taxa that have been assigned to Pleurobemini results in several nomenclatural changes. The type species of *Quincuncina* and *Lexingtonia* place within *Fusconaia*. *Eurynia*, *Sintoxia*, *Pleuronaia*, and *Rotundaria* deserve recognition as subgenera or genera based on molecular, conchological, and anatomical features; new genera will probably be needed for some additional taxa such as *Pleurobema collina*.

Within Pleurobemini, three previously synonymized genera are recognized. *Eurynia* applies to "*Elliptio*" *dilatata*. *Sintoxia* is the oldest available subgenus for the "*Pleurobema*" *cordatum* group (*cordatum*, *plenum*, *riddellii*, *rubrum*, *sintoxia*). *Pleuronaia* includes "*Fusconaia*" *barnesiana* (Lea, 1838), "*Lexingtonia*" *dolabelloides* (Lea, 1840), and "*Pleurobema*" *gibberum* (Lea, 1838). Several species currently assigned to Pleurobemini are removed from the tribe; many also require nomenclatural changes. *Fusconaia apalachicola*, *F. ebenus*, and *F. rotulata* receive the new genus *Reginaia* herein. *Reginaia* is not clearly associated with any particular tribe within Ambleminae. *Fusconaia succissa*, *Cyclonaias tuberculatus*, and the *Quincuncina infucata* complex belong in the "*Quadrula*" *pustulosa* group. A convoluted nomenclatural history restores *Rotundaria* as the oldest genus name for the *pustulosa* group. *Uniomerus* also shows molecular affinity to Quadrulini rather than Pleurobemini.

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Mantle display and glochidia release behaviors of five quadruline freshwater mussel species (Bivalvia: Unionidae)

Bernard E. Sietman[1], **J. Mike Davis**[2], **and Mark C. Hove**[3]

[1] Minnesota Department of Natural Resources, 500 Lafayette Road, St. Paul, Minnesota 55155, U.S.A.

[2] Minnesota Department of Natural Resources, 1801 South Oak Street, Lake City, Minnesota, 55041 U.S.A.

[3] Department of Fisheries, Wildlife, and Conservation Biology, University of Minnesota, 1980 Folwell Avenue, Saint Paul, Minnesota, 55108 U.S.A.

Correspondence, Bernard E. Seitman: bernard.sietman@state.mn.us

Abstract: Diverse strategies have evolved in freshwater mussels to promote the transfer of their parasitic larvae to host fish. Among these, modification of the mantle as a host attracting lure has been well-documented in the Tribe Lampsilini, but only recently reported in the Tribe Quadrulini. Here we describe mantle modifications and glochidia release behaviors in five quadruline species, including members of the *Quadrula quadrula* (Rafinesque, 1820), *Q. pustulosa* (Lea, 1831), and *Q. metanevra* (Rafinesque, 1820) species groups. Displays were motionless and consisted of inflated mantle tissue surrounding the excurrent aperture. Gross display morphology was largely variable among species: *Q. fragosa* (Conrad, 1835) and *Tritogonia verrucosa* (Rafinesque, 1820) had relatively large, uniquely shaped displays; *Cyclonaias tuberculata* (Rafinesque, 1820) and *Q. pustulosa* had smaller, stomate-shaped displays; and *Q. metanevra* had a diminutive, polyp-like display. *Cyclonaias tuberculata* exhibited a bimodal host infection strategy where individuals had either a mantle display or released a gelatinous conglutinate. *Quadrula pustulosa* and *Q. metanevra* expelled glochidia in a forceful burst when their displays were touched. Quadruline mantle displays do not clearly mimic identifiable aquatic organisms suggesting they may represent non-specific food items to their fish hosts.

Key words: fish lure, life history, *Quadrula*, *Cyclonaias*, *Tritogonia*

The freshwater bivalve family Unionidae is a group of mostly riverine mollusks that have their greatest diversity in eastern North America (Graf and Cummings 2007, Bogan 2008). Most unionids have a life cycle in which their larvae (glochidia) are obligate parasites on vertebrate hosts, usually attaching to the gills or fins of fish where they may undergo transformation into free-living juveniles (Kat 1984, Wächtler *et al.* 2001). Many species have evolved impressive strategies to facilitate transfer of glochidia to the host, usually involving some form of food mimicry. Well-known examples include: mantle modifications "lures or displays" that resemble fish or invertebrates (Barnhart and Roberts 1997, Haag *et al.* 1999, Haag and Warren 2003, Allen *et al.* 2007, Rypel 2008), packaging of glochidia into conglutinates resembling insects or worms (Barnhart and Roberts 1997, Watters 1999, Jones and Neves 2002, Haag and Warren 2003), and minnow-like superconglutinates (Haag *et al.* 1995, Hartfield and Butler 1997). In most cases, the morphology of these lures appear well-suited to the feeding habits of the host.

Multiple genera in the Tribe Lampsilini use mantle displays as a host attraction strategy (Zanatta and Murphy 2006, Barnhart *et al.* 2008); however, these structures were only recently recognized in the Tribe Quadrulini (Barnhart *et al.* 2008). In that study, *Quadrula cylindrica* (Say, 1817), *Q. fragosa* (Conrad, 1835), *Q. pustulosa* (Lea, 1831), and *Tritogonia verrucosa* (Rafinesque, 1820) were shown to have mantle displays (termed the mantle magazine), and some aspects of glochidia brooding and release behaviors were discussed. As currently recognized, the genus *Quadrula* Rafinesque, 1820 is composed of three distinct clades, the *quadrula*, *pustulosa*, and *metanevra* species groups (Simpson 1900, Frierson 1927, Serb *et al.* 2003). Host fishes vary among these groups, with the *quadrula* and *pustulosa* species groups using catfishes (Ictaluridae) (Howard 1914, Howells 1997, Hove *et al.* 2001, Haag and Warren 2003, Steingraeber *et al.* 2007) and the *metanevra* species group using minnows (Cyprinidae) (Yeager and Neves 1986, Yeager and Saylor 1995, Crownhart *et al.* 2006). Assuming that mantle displays in these unionids facilitate glochidia transfer to the host, their morphology and brooding behaviors should be adapted to attract these disparate host groups.

Furthermore, there has been renewed debate regarding the classification of some quadruline species, such as the proposed placement of *Tritogonia* Agassiz, 1852 within *Quadrula* (Serb *et al.* 2003). *Cyclonaias* Pilsbry in Ortmann and Walker, 1922 is presumed to be a valid monotypic genus (Graf and Cummings 2007, Williams *et al.* 2008, Watters *et al.* 2009), yet its phylogenetic placement is uncertain (Heard and Guckert 1970, Serb *et al.* 2003, Campbell *et al.* 2005). There is further disagreement whether the three species groups should be lumped within *Quadrula* (Serb *et al.* 2003, Williams *et al.* 2008) or treated as distinct genera (Graf and

Cummings 2007, Watters *et al.* 2009). A better understanding of mantle display morphology and glochidia brooding behaviors within the Quadrulini could be helpful in resolving these questions.

In this study, we add to previous work by describing gross structure and phenology of mantle displays and glochidia release behaviors of five quadruline species, including members of the three primary quadruline clades. Since mantle display and conglutinate morphology presumably evolved to facilitate transfer of mussel larvae to its host(s) (Barnhart *et al.* 2008), and these structures apparently are adapted to suit the feeding habits of the host fish, we discuss our findings in context of known host associations.

MATERIALS AND METHODS

We studied unionid brooding behaviors in the St. Croix River at Interstate State Park, Minnesota and Wisconsin, U.S.A. (45° 23′ 36″N, 92° 39′ 47″W) between April and September, from 2004 to 2008. This reach of the St. Croix holds a regionally diverse assemblage of over 30 unionid species (Hornbach 2001), many of which are locally or globally rare. Hornbach *et al.* (1996) provides general habitat and unionid assemblage characteristics of the study area. We searched for displaying unionids every 1–4 weeks as time and conditions allowed, mostly at night using hand-held underwater lights. Diel patterns of displaying behavior were not examined in this study (but see Hove *et al.* 2011). Water clarity ($\approx$ 1 m visibility) was suitable to easily observe unionids *in situ*. Water depth varied during the study, and we used SCUBA when depth was $>$ 0.6 m, and a mask and snorkel otherwise. When a displaying mussel was encountered, we recorded underwater digital photographs (with flash) or video, then examined the specimen to confirm its identification and released it. We did not measure display dimensions, but estimated relative size compared to shell dimensions from photographs and video. Displays were categorized as either fully or weakly inflated by observing multiple displays over several years during the brooding period. We did not count displaying individuals because of difficulty tracking the area covered by up to 5 persons at night.

To simulate a fish encounter and elicit the release of glochidia, we touched or tapped displays with a fingertip while recording video. We captured released glochidia with a fine mesh net or plastic bag when possible, or extracted glochidia from a subset of displaying females. We examined glochidia with a light microscope, and considered them viable if they had fully formed valves and snapped shut after adding table salt to the water (Zale and Neves 1982).

Mean daily water temperature for observation dates was obtained from USGS gauging station 05340500 at St. Croix Falls, Wisconsin, approximately 2.4 km upstream of our study area. Voucher specimens of each species from the study area, although usually not of the displaying individuals (because of their protected status), are held in the University of Minnesota James Ford Bell Museum of Natural History.

RESULTS

Representative displays for the five mussel species are shown in Fig. 1. Additional photographs showing variation among individuals or different viewing angle, and video footage of distinct glochidia release behaviors are posted online (http://mndnr.gov/mussels/quadrula).

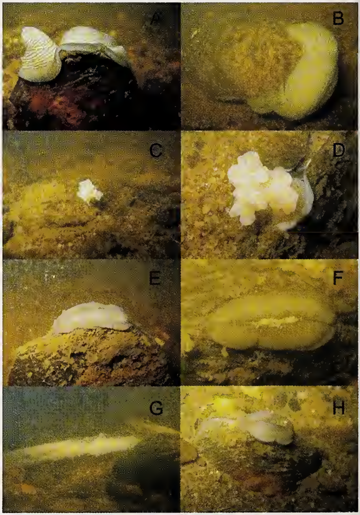

Figure 1. Typical quadruline mantle displays. **A**, *Quadrula fragosa* lateral view; **B**, *Tritogonia verrucosa* posterior-lateral view; **C**, *Quadrula metanevra* lateral view, and **D**, dorsal view close up; **E**, *Cyclonaias tuberculata*, lateral view; **F**, dorsal view close up, with glochidia clearly visible in the center of the display, and **G**, conglutinate, lateral view; **H**, *Quadrula pustulosa*, dorsal-lateral view; material in center of the display is sand.

Cyclonaias tuberculata

Display

Brooding female *Cyclonaias tuberculata* produced a relatively small, stomate-shaped display, by inflating the mantle region around the excurrent aperture (Figs. 1E–F). Displaying females emerged from the substrate such that approximately half of the posterior shell was exposed. Display length was about 35–40% of dorsal-ventral shell height, and the swollen mantle often enveloped a small portion of the posterior shell margin. Displays were bluish-grey with faint, dark speckles that frequently aligned to form horizontal lines. We observed displays from June to the beginning of August when daily water temperature ranged from 19 to 27 °C (Fig. 2), although within the brooding period individuals displayed for only about a month.

Glochidia release

Non-displaying *Cyclonaias tuberculata* released a gelatinous conglutinate that trailed from the excurrent aperture (Fig. 1G) and swayed with the water current (see video http://mndnr.gov/mussels/quadrula). Conglutinates were amorphous and transparent, varied in size from about 5 to 20 cm in length, and embedded glochidia were clearly visible. Some detached conglutinates were also observed lying on the substrate or tumbling on the stream bottom with the current. Conglutinates and mantle displays occurred at similar water temperatures and time of the year (Fig. 2), but these behaviors did not occur simultaneously within individuals. Some individuals had loosely assembled glochidia clearly visible between the swollen tissues of the mantle magazine (Fig. 1F). After several attempts, no displaying individuals expelled glochidia in response to the touch of a finger.

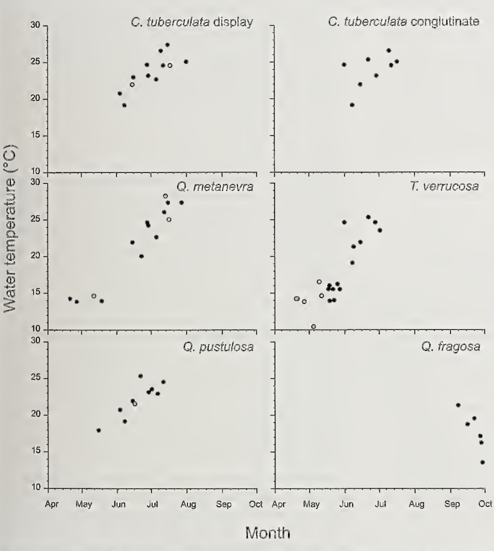

Figure 2. Phenology of displaying behavior for five quadruline mussel species in the St. Croix River, Minnesota and Wisconsin. Each point represents a date when one or more displaying individuals were observed. Black symbols are fully inflated displays; shaded symbols are weakly inflated displays (see text). *Cyclonaias tuberculata* conglutinate data include observations when a conglutinate was attached to the mussel or found detached. Water temperature data were retrieved online from the St. Croix Falls USGS gauging station 05340500 (http://waterdata.usgs.gov/wi/nwis/rt).

Quadrula fragosa

Display

Brooding female *Quadrula fragosa* had a large display that extended from the excurrent aperture to the posterior limit of the dorsal shell margin (Fig. 1A). Displaying females emerged from the substrate such that about 50–70% of the shell was exposed, and valves were oriented vertically with the anterior end remaining buried in the substrate. The most prominent feature of the display was a large conical knob-like structure with a rounded tip that formed the mantle magazine. This structure originated from mantle tissue next to the incurrent aperture, which was at the knob base. A slit, evidently composed of the excurrent aperture, ran from the tip of the knob until it connected with the incurrent aperture. Dorsal to the knob, the distended mantle formed broad wings that draped over the shell margin, with a v-shaped furrow at the midpoint where the wings joined. Relative size of displays varied among individuals. Displays were whitish to blue-grey, and the surface of the knob had well-defined irregular horizontal stripes with interspersed speckles. Stippling on the wings formed fine striae, which were oriented perpendicular to the furrow. A few small tubercles occurred around the knob or in the furrow of three individuals. The display of two individuals appeared to be damaged. The wing margins were ragged and white, as if tissue had been torn away, and the knob tips were similarly discolored.

Female *Quadrula fragosa* displayed from mid to late September when daily water temperature ranged from 16.2 to 19.5 °C (Fig. 2).

Glochidia release

In the laboratory, a single *Quadrula fragosa* with a partially inflated display released conglutinates with mature glochidia, but the release was not a forceful expulsion (M. C. Hove pers. obs.). Conglutinates were thin and white, approximately 5 mm wide by 10 mm long, tapered at both ends, and fragmented somewhat easily but were not as fragile as *Tritogonia verrucosa* conglutinates. Because of the endangered status of this species, we touched the mantle display of just two specimens in the field and neither released glochidia. Additionally, on several occasions displaying animals touched the container walls during transport to the laboratory but did not release glochidia.

Quadrula metanevra (Rafinesque, 1820)

Display

The display of *Quadrula metanevra* was the smallest among the species studied and somewhat resembled a coral polyp (Figs. 1C–D). As the display developed, tissue surrounding the excurrent aperture inflated and became progressively wrinkled or folded, sometimes developing a short stalk at the base. The degree of wrinkling, which affected overall display shape, was variable among individuals. Relative display length was about 15–25% of dorsal-ventral shell height. Displays were usually whitish, occasionally light pink or light orange, with irregular faint mottling. Displaying females were oriented in the substrate similar to non-displaying individuals, being mostly or completely buried.

Quadrula metanevra displayed for about 3 months within a year, from mid-April to late July, when daily water temperature ranged from 13.9 to 24.6 °C (Fig. 2). This was the longest brooding period among species in this study.

Glochidia release

Five *Quadrula metanevra* expelled loose glochidia in forceful bursts when the displays were touched with a fingertip. Mature glochidia were captured from one such release. One individual released glochidia before it was touched, suggesting the observer nearby stimulated the release (see video http://mndnr.gov/mussels/quadrula).

Quadrula pustulosa

Display

Quadrula pustulosa's mantle display was very similar in appearance to *Cyclonaias tuberculata* (Fig. 1H). Relative display size varied among individuals, ranging between 30–50% of dorsal-ventral shell height. Color was light tan or grayish with faint or well-defined speckles that frequently aligned to form horizontal lines. Displaying females usually emerged from the substrate such that approximately 25 to 40% of the posterior shell was exposed. We observed displays for up to 2 months within a year, from mid-May to mid-July, when daily water temperature ranged from 17.9 to 25.3 °C (Fig. 2).

Glochidia release

Four *Quadrula pustulosa* expelled glochidia in forceful bursts when the displays were touched with a fingertip (see video http://mndnr.gov/mussels/quadrula). Glochidia were loose or weakly bound in a white material that readily fragmented, and was less cohesive than the gelatinous conglutinate produced by *Cyclonaias tuberculata*.

Tritogonia verrucosa

Display

Brooding *Tritogonia verrucosa* had a large fleshy display that extended around the entire curve of the posterior shell margin, from the dorsal-posterior point where the shell begins to slope, ventrally to where the posterior ridge meets the shell margin, and encompassed both the incurrent and excurrent apertures (Fig. 1B). Females emerged from the substrate in mid-April to early May, lying on their side or with the ventral margin down. After emergence, we observed displays in the early stages of development when average daily water temperature was < 13.5 °C. Fully inflated displays were seen between mid-May to early July, for 4.5 to 6.5 weeks within a brooding season, when daily water temperature ranged from 13.9 to 25.3 °C. The margin of the left and right mantle edges were finely serrated and frequently overlapped along the thickest region of the display. Displays were whitish, occasionally with varying degrees of bluish shading. The surface was covered with shallow, irregularly spaced, bluish or grey colored groves, lines, and pits, which gave displays a coarse or corrugated appearance. These characters, as well as display size, were variable among individuals. *Tritogonia verrucosa* was the most commonly observed displaying quadruline at our study site, and there were noticeable within-year peaks of displaying frequency indicating synchronous behavior.

Glochidia release

Tritogonia verrucosa released loose glochidia or broken and whole conglutinates but did not forcefully expel glochidia in response to touch. Broken and whole conglutinates had an elongate rectangular outline, were 3 to 25 mm long and 2 to 3 mm wide, light yellow to light brown, and fragmented easily.

DISCUSSION

Display morphology and host associations

Host attraction strategies used by unionids generally involve one of two types of food mimicry, mantle modifications intended to lure host fish to the brooding mussel (Barnhart and Roberts 1997, Haag *et al.* 1999, Allen *et al.* 2007, Rypel 2008) or the release of conglutinates that resemble host food items (Haag *et al.* 1995, Barnhart and Roberts 1997, Hartfield and Butler 1997, Watters 1999, Jones and Neves 2002, Haag and Warren 2003). These features presumably evolved to suit the feeding habits of the host or a suite of host species, and are adapted to attract and facilitate glochidia transfer to the host (Zanatta and Murphy 2006). The hypothesis that quadruline mantle displays function as host-attracting lures is supported by the presence of mature glochidia in these individuals.

Size and shape of quadruline mantle displays varied among most species in our study; however, all were composed of inflated mantle tissue surrounding the excurrent aperture (as well as the incurrent aperture in *Tritogonia verrucosa*). This differs from described lampsiline mantle displays which are positioned anterior to the incurrent aperture along the ventral or posterior-ventral shell margin (Kraemer 1970, Haag *et al.* 1999, Allen *et al.* 2007). While their function in attracting host fish is likely similar, the distinct position of mantle displays in the Lampsilini and Quadrulini and the observation that these taxa are not sister lineages (Campbell *et al.* 2005) indicates that mantle displays evolved independently in these two clades.

We suspect that variation in display morphology among quadruline species is suited to the host fish, but it is unclear what food items are being mimicked. In our study area, channel catfish (*Ictalurus punctatus*) is likely the principal host for *Cyclonaias tuberculata*, *Quadrula fragosa*, and *Q. pustulosa* (Howard 1914, Hove 1997, Hove *et al.* 2001, Steingraeber *et al.* 2007) and flathead catfish (*Pylodictis olivaris*) for *Tritogonia verrucosa* (Howells 1997, Hove *et al.* 2011). Various minnows (Cyprinidae) are suitable hosts for *Q. metanevra* (Crownhart *et al.* 2006) and other species within the *metanevra* species group (Yeager and Neves 1986, Yeager and Saylor 1995).

In species with catfish hosts, pale color and absence of active movement indicate these lures might mimic dead or moribund fish, or perhaps live, moribund, or dead unionids. Channel and blue catfish (*Ictalurus furcatus*) will consume unionids, fingernail clams, and snails (Forbes 1888, Brown and Dendy 1961, Lewis 1976, Griswold and Tubb 1977), possibly in abundance (reviewed by Graham 1999), but they are not known to eat mature quadruline mussels. It is also possible these lures do not visually mimic a particular food item because selection on lure morphology has not been as strong with these species. Channel and blue catfish are omnivores, consuming a variety of food items including live fish (Bailey and Harrison 1948, Griswold and Tubb 1977, Pflieger 1997, Graham 1999, Edds *et al.* 2005), whereas flathead catfish primarily consume live prey: invertebrates when young and then turning piscivorous (Brown and Dendy 1961, Layher and Boles 1980, Pflieger 1997). Therefore, quadruline displays could simply represent a generalization of a subset of the many available food items these catfish consume, including live prey. Catfish also use taste and olfactory receptors to find food (Caprio 1978) so there could be a chemical signal associated with these lures (Barnhart *et al.* 2008, Hove *et al.* 2011), a hypothesis that remains to be tested. Although we did not record any fish-display interactions, Barnhart *et al.* (2008) observed channel catfish attack the display of *Tritogonia verrucosa*, even though this fish species is not a suitable host based on testing done by Hove *et al.* (2011). We encountered two seemingly damaged *Quadrula fragosa* displays which could have resulted from fish attacks.

Orientation of displaying females varied among species. Species that use catfish as hosts emerged from the substrate to varying degrees, and therefore, do not conceal themselves while brooding glochidia, a behavior Haag *et al.* (1995) observed in the lampsiline *Hamiota perovalis* (Conrad, 1834) which uses a superconglutinate lure. This behavior was most pronounced in *Tritogonia verrucosa* and *Quadrula fragosa*, species with the largest displays in our study, but was also noticeable in *Cyclonaias tuberculata* and to a lesser extent in *Q. pustulosa*. Presumably this behavior has evolved to attract nocturnally foraging ictalurids. In contrast, *Q. metanevra* remains mostly buried while displaying its diminutive, pale colored lure. Though small, this display is conspicuous and presumably resembles a small generic food item to sight-feeding minnows (Pflieger 1997). Remaining buried might improve the display's effectiveness by concealing the shell, a behavior we have also seen in *Lampsilis higginsii* (Lea, 1857), and to a lesser degree in *L. cardium* Rafinesque, 1820, which display a minnow-like lure to attract piscivorous hosts. The excurrent aperture of the closely related *Q. cylindrica* (also a member of the *metanevra* species group) is not inflated as in *Q. metanevra*, but is brightly-colored (Barnhart *et al.* 2008). These displays presumably elicit feeding encounters from foraging cyprinids, as do the small reddish or white conglutinates released by *Fusconaia* Simpson, 1900 and *Pleurobema* Rafinesque, 1819 (Tribe Pleurobemini) (Haag and Warren 2003).

Glochidia release behaviors

Quadruline unionids transfer glochidia from the marsupial demibranchs to the mantle magazine (Barnhart *et al.* 2008), a storage pocket formed by the swollen mantle (display) surrounding the excurrent aperture, where they are held before release. From the mantle magazine, transfer of glochidia to the host occurs by reflexive release (forceful expulsion of glochidia), extraction by the host, or conglutinate release (Barnhart *et al.* 2008).

Cyclonaias tuberculata is one of the few unionids that exhibit multiple host infection behaviors, in this case, a mantle display and amorphous conglutinate. This bimodal system is analogous to the lampsiline genus *Hamiota* Roe and Hartfield, 2005, where the female has both a mantle display and releases a superconglutinate (Hartfield and Butler 1997). *Cyclonaias tuberculata* gradually released gelatinous conglutinates with embedded glochidia, allowing them to trail behind the mussel and sway in the current, eventually detaching from the mussel, an effect also seen in *Hamiota* (Haag *et al.* 1995, Hartfield and Butler 1997). As with mantle displays, these conglutinates could function as a visual or chemical lure. Similar to *Hamiota*, we suspect the entire contents of the marsupia are released based on our observations, but this assumption needs to be examined further. In *Hamiota*, however, some individuals use both strategies at different times during the brooding period (Hartfield and Butler 1997). This is apparently not the case with *C. tuberculata* which likely use one method or the other, at least during a brooding period, because glochidia of displaying individuals were loosely assembled in the mantle magazine, whereas glochidia of conglutinate producers were embedded in a gelatinous mucous. Zanatta *et al.* (2007) discussed several hypotheses for the existence and maintenance of polymorphic lure morphologies in *Lampsilis fasciola* Rafinesque, 1820, which could also apply to the strategies seen in *C. tuberculata*.

Structured conglutinates are not used by quadrulines as primary host infection strategy as far as we know, although they are often released prematurely with eggs or undeveloped glochidia when disturbed (Lefevre and Curtis 1912). We have seen *Quadrula fragosa*, *Q. quadrula* (Rafinesque, 1820), and *Tritogonia verrucosa* release conglutinates with mature glochidia on occasion, with those of *T. verrucosa* being more fragile and easily fragmented. In contrast, several other amblemine genera have functionally structured conglutinates (Watters 2008) as their primary host infection strategy, but apparently do not use reflexive release. It is likely that physical or proximal contact with displays by foraging fish stimulates reflexive glochidia release. We recorded several instances of reflexive release among displaying *Q. metanevra* and *Q. pustulosa* in response to physical stimuli. Barnhart *et al.* (2008) reported reflexive release in *Q. pustulosa*, *Q. cylindrica*, and *Cyclonaias tuberculata*, although in our study we did not elicit a reflexive release from displaying *C. tuberculata*. The proportion of glochidia in a brood ejected during a release is unknown. Reflexive release of glochidia could potentially infest multiple fish during a single event, particularly *Q. metanevra* parasitizing schooling minnows. Vicentini (2005) reported a type of reflexive release behavior in *Unio crassus* Philipsson in Retzius, 1788 which spout a fine stream of water with glochidia into the air, evidently attracting fish to the splashing water.

In contrast, *Quadrula fragosa* and *Tritogonia verrucosa* apparently rely on host fish to extract glochidia when they attack the display (Barnhart *et al.* 2008), a strategy similar to some lampsilines (Haag and Warren 1999). In the laboratory or field, we occasionally observed *Q. fragosa* and *T. verrucosa* gradually release whole or broken conglutinates unsolicited, but not via reflexive release. These releases could be either incidental spillage from the mantle magazine or an alternative strategy to infest the host. Barnhart *et al.* (2008) postulated that species using both mantle lures and releasing conglutinates potentially could contact a wider range of hosts, and it is conceivable that catfish, perhaps smaller individuals, become infested by consuming these conglutinates.

Brooding characteristics described in this study represent a diverse set of morphological and behavioral adaptations to facilitate glochidia transfer to host fish, and exemplifies the diversity within the Quadrulini. Mantle display, conglutinate morphology, and glochidia release behaviors can be informative characters in phylogenetic studies (Zanatta and Murphy 2006, Watters 2008). While our results offer no conclusive insights into evolutionary relationships among quadruline species, because of, in part, insufficient species representation, the distribution of display and glochidia release character states corroborate aspects of quadruline phylogeny. For example, the similar display morphology of *Cyclonaias tuberculata* and *Quadrula pustulosa* support Campbell *et al.*'s (2005) placement of *Cyclonaias* within the Quadrulini and its apparent relationship with the *pustulosa* species group. Similarly, the relatively diminutive mantle displays, use of reflexive glochidia release, and unique host association with Cyprinidae corroborate the relatively close evolutionary relationship between *Q. metanevra* and *Q. cylindrica* (Serb *et al.* 2003).

As mantle display morphology and glochidia brooding and release behaviors are described for additional quadruline species, these character states, as well as glochidia morphology and host fish associations, will enhance our understanding of unionid reproductive biology and may help resolve questions of phylogeny and classification.

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Early life history of the winged mapleleaf mussel (*Quadrula fragosa*)

Mark C. Hove[1,2], Mark T. Steingraeber[3], Teresa J. Newton[4], Dave J. Heath[5], Carrie L. Nelson[1], Jennifer A. Bury[1], Jennifer E. Kurth[1], Michelle R. Bartsch[4], Whitney S. Thorpe[1], Marissa R. McGill[1], and Daniel J. Hornbach[2]

[1] Department of Fisheries, Wildlife and Conservation Biology, University of Minnesota, 1980 Folwell Avenue, Saint Paul, Minnesota 55108, U.S.A.

[2] Biology Department, Macalester College, 1600 Grand Avenue, Saint Paul, Minnesota 55105, U.S.A.

[3] US Fish and Wildlife Service, La Crosse Fishery Resource Office, 555 Lester Avenue, Onalaska, Wisconsin 54650, U.S.A.

[4] US Geological Survey, Upper Midwest Environmental Sciences Center, 2630 Fanta Reed Road, La Crosse, Wisconsin 54603, U.S.A.

[5] Wisconsin Department of Natural Resources, 3550 Mormon Coulee Road, La Crosse, Wisconsin 54601, U.S.A.

Correspondence, Mark C. Hove: mark_hove@umn.edu

Abstract: Early life history information on the federally endangered winged mapleleaf (*Quadrula fragosa* (Conrad, 1835)) mussel is needed by natural resource managers to improve conservation and propagation programs. We conducted four studies to obtain some of this information. First, we observed *Q. fragosa* in the St. Croix River, Wisconsin between 1997–2010 and found females brood larvae (glochidia) for a unique period, between 8 September to 8 October at water temperatures ranging between 15–21 °C. Second, we tested 67 fish species and *Necturus maculosus* for their ability to transform glochidia into juveniles. Nearly 30,000 juvenile *Q. fragosa* were produced, but only on *Ictalurus furcatus* and *I. punctatus*. Unlike most mussel species, *Q. fragosa* glochidia grew 3 to 4-fold while attached. Third, using scanning electron microscopy we were able to distinguish *Q. fragosa* glochidia height and length from six other mussel species that also produce small glochidia (<120 µm in height). Finally, we recovered 535 juveniles from 15 naturally infested fish species to determine if any were *Q. fragosa* based on glochidia morphology. We identified one juvenile from an *I. punctatus* as a *Q. fragosa*. To improve conservation efforts we suggest monitoring not only *Q. fragosa* beds but also associated *Ictalurus* populations, whose ranges may extend beyond mussel populations.

Key words: Unionidae, freshwater mussel, brooding period, glochidia hosts

The winged mapleleaf mussel (*Quadrula fragosa* (Conrad, 1835)) was listed as a federally endangered species in 1991 as a result of range reduction, small population sizes, and poor recruitment (U.S. Fish and Wildlife Service 1991). The recovery plan for *Q. fragosa* defines recovery as the existence of at least five populations capable of long term persistence (U.S. Fish and Wildlife Service 1997). While this species was historically found in at least 34 rivers in 12 states, the only known reproducing population at the time of listing was in the St. Croix River, Wisconsin, with a possible population in the Kiamichi River, Oklahoma (U.S. Fish and Wildlife Service 1997). Since 1996, another reproducing population has been found in the Saline River, Arkansas (W. R. Posey, pers. comm.), and populations have been found in the Ouachita River, Arkansas (Posey *et al.* 1996, Serb and Harris 2003) and Bourbeuse River, Missouri (M. C. Barnhart, pers. comm.).

The findings of additional *Quadrula fragosa* populations have prompted research that has provided new insights into the biology of this species. For example, historical records describe the species as living in large streams in mud, mud-covered gravel, or gravel (Wilson and Clark 1914, Ortmann 1924, Baker 1928). Yet, *Q. fragosa* in the St. Croix River is found in areas with high water velocity in gravel, sand or rubble substrates (Hornbach *et al.* 2010). *Quadrula fragosa* is also usually found associated with dense and diverse communities of other mussels (Hornbach *et al.* 1996). These data show there is much to learn about the life history of this imperiled species.

Since most freshwater mussels require a host fish to complete their life cycle, identification of host fish species is needed by resource managers to propagate rare mussel species. For example, with knowledge of host fishes, federally listed Higgins eye (*Lampsilis higginsii* (I. Lea, 1857)) has been cultured and restocked into portions of the upper Mississippi River (Wege 2001) and several other facilities are now culturing and stocking threatened and endangered species into their historical ranges (Henley *et al.* 2001, Barnhart 2006). The lack of information on host fish species is identified as a limiting factor in many endangered freshwater mussel species recovery plans.

Host fish are usually identified either by collecting fish and identifying naturally encysted glochidia or by exposing many fish species to glochidia in the laboratory and then isolating each species until metamorphosis occurs (Hove *et al.* 2000, Khym and Layzer 2000). Taken separately, each method has its limitations. In the wild, fish with attached glochidia may slough glochidia before metamorphosis and freshly released juveniles from these fish must be carefully scrutinized for accurate identification (Kennedy and Haag 2005, Allen *et al.* 2007). In the laboratory, metamorphosis may occur on some species that may not naturally come in contact with glochidia. Early screening efforts at the University of Minnesota (UMN) identified channel catfish (*Ictalurus punctatus*) as a suitable *Quadrula fragosa* host (Hove *et al.* 2002).

Development of embryonic and encapsulated glochidia is thought to be temperature-dependent. For example, the development of embryonic duck mussel (*Anodonta anatina* (Linnaeus, 1758)) glochidia was influenced by temperature, brooding glochidia at the time of highest water temperature – presumably because this results in a high output of glochidia at a low energetic cost (Jokela *et al.* 1991). Higher water temperature generally shortens the period of metamorphosis (Roberts and Barnhart 1998, Gray *et al.* 2002). The time required for metamorphosis of *Quadrula fragosa* glochidia is predictable based on water temperature (Steingraeber *et al.* 2007). However, the amount of time glochidia are brooded in the marsupial gills is also important in regulating the length of time to metamorphosis (Khym and Layzer 2000).

Since an understanding of the early life history of *Quadrula fragosa* is crucial for propagation efforts to protect and restore this species, the objectives of this study were to: (1) describe *Q. fragosa* brooding period, (2) determine suitable hosts for *Q. fragosa* glochidia, (3) compare the morphology of *Q. fragosa* glochidia with other mussel species in the St. Croix River, and (4) collect juvenile *Q. fragosa* from naturally infested fishes.

MATERIALS AND METHODS

Brooding behavior

We studied *Quadrula fragosa* brooding behaviors in the St. Croix River and at the UMN's Wet Laboratory between 1997–2010. Between May to November 1997 and April to October 1998 we collected 6–25 mussels bimonthly during daylight hours using SCUBA and snorkel equipment and checked them for gravidity. Gravid animals were identified as those that had gills swollen with embryos, glochidia or conglutinates (glochidia aggregations within a gelatinous matrix) or held conglutinates beneath a mantle magazine (Barnhart *et al.* 2008). We also observed mussels approximately biweekly between September to October 2000–2010 while we gathered brooding females for U.S. Fish and Wildlife Service (USFWS) juvenile propagation efforts. During this time we increased the probability of finding *Q. fragosa* by creating several aggregations of 10–15 adults in mid-summer before the suspected reproductive period in August. Between 2000–2010 we terminated searches for brooding mussels when the propagation quota was met so the end of the brooding period was likely missed during many years. We identified mussels using Sietman (2003) and estimated mussel age by counting external annuli.

Host suitability trials

To initiate host suitability trials we obtained glochidia from brooding *Quadrula fragosa* from the St. Croix River, Minnesota. These mussels were held 1–2 wk in 0.5–1 L beakers placed in either flow-through or static 40 L aerated aquaria containing well water at temperatures ± 2 °C of river temperatures. The methods used to examine glochidia are described in Hove *et al.* (2011). Viable glochidia were exposed to potential hosts within 12 h of release. We estimated the number of glochidia used for some exposures by placing glochidia in a flask containing a known volume of water and suspending the contents. From this slurry we quickly removed a 2 mL aliquot with a pipette and preserved it with 70% ethanol. These glochidia were later enumerated using a dissection microscope and cross polarized light. For most trials we used glochidia from a single female but glochidia from up to three females were combined and exposed to some fish species. Due to limited numbers of brooding females, we could not run replicate trials with all fishes from various sources under different thermal regimes. We calculated the percentage of metamorphosed glochidia = (no. juveniles recovered/no. glochidia recovered x 100) for some host trials.

We followed standard protocols (Zale and Neves 1982, Hove *et al.* 2000) to determine suitable hosts and obtain juvenile mussels from naturally infested fishes. To obtain potential hosts we primarily collected them from central and southern Minnesota, or they were provided by private, state, or federal hatcheries. These animals were held for > 2 months before use in host suitability trials, which we initiated in September to October 1998–2000 and 2002–2003. Generally, we used standard methods described in Hove *et al.* (2011) to infest fish with glochidia. A small number of fishes (flathead catfish (*Pylodictus olivaris*) and slender madtom (*Noturus exilis*) were anesthetized with Finquel® (tricaine methanesulfonate) and glochidia were pipetted directly onto their gills.

Host suitability trials were conducted under two thermal regimes. We conducted most host suitability trials at the lowest temperature available at our UMN laboratory (11 °C) to mimic water temperatures encountered by this species during glochidia release in October (10 ± 3 °C). However, we had to maintain fish for 6–9 months at this temperature to facilitate glochidia metamorphosis and the lower temperatures presumably facilitated several outbreaks of "Ich" (*Ichthyophthirius multifilis*) in which many *Ictalurus punctatus* died We used a combination of wild and hatchery-reared fishes and tested

67 fish species and the mudpuppy [a salamander] (*Necturus maculosus*) in the low temperature trials. We conducted trials at warmer temperatures (19–20 °C) at the Upper Midwest Environmental Sciences Center (UMESC) to increase the likelihood of *I. punctatus* survival, rapidly test different species of host fish candidates, and mimic temperature encysted *Quadrula fragosa* might experience during September in the St. Croix River (18 ± 3 °C). We used hatchery-reared blue catfish (*I. furcatus*) and channel catfish, as well as wild tadpole madtom (*Noturus gyrinus*), *N. exilis*, and *Pylodictus olivaris* for the warm water trials.

We held animals with attached glochidia in aquaria until glochidia were sloughed or juvenile mussels were released. Depending on the size of fish we held animals at a density of 1–17/aquarium. We fed fish held at 11 °C 1–2 times per wk and those at 15–22 °C 3 times per wk. Small fish (*e.g.* cyprinids, etheostomids, catostomids) were fed frozen brine shrimp (*Artemia* sp.) or blood worms (larval chironomids) and piscivorous fish fathead minnows (*Pimephales promelas*). At the UMESC we conducted host suitability trials similarly, except that test fish were held in 38 L aquaria fitted with false bottoms (plastic screen with 25 mm^2 openings) positioned 3.5 cm above the aquarium floor and were fed small rainbow trout (*Oncorhynchus mykiss*) once per wk.

We followed methods described in Hove *et al.* (2011) to sieve siphonate through a 45 μm sieve, search for glochidia, identify juveniles, and terminate host suitability trials. Unionid, fish, and amphibian nomenclature follows Turgeon *et al.* (1998), Robins *et al.* (1991), and Crother *et al.* (2000), respectively.

Glochidia morphology

To help us identify juvenile mussels released from naturally infested fishes we collected glochidia from seven St. Croix River mussel species that produce small glochidia (height <120 μm) (spectaclecase (*Cumberlandia monodonta* (Say, 1829)), fragile papershell (*Leptodea fragilis* (Rafinesque, 1820)), *Quadrula fragosa*, mapleleaf (*Q. quadrula* (Rafinesque, 1820)), pistolgrip (*Tritogonia verrucosa* (Rafinesque, 1820)), fawnsfoot (*Truncilla donaciformis* (I. Lea, 1828)), and deertoe (*T. truncata* Rafinesque, 1820) (Surber 1912, 1915). These glochidia were obtained from gravid females from the St. Croix and Mississippi rivers, Minnesota and preserved in 95% ethanol. We washed glochidia with 100% ethanol and mounted them on conductive tape on scanning electron microscope specimen stubs. To coat specimens with gold we used a Fullam Sputter Coater and then viewed them with a Hitachi S3500N variable pressure scanning electron microscope with Quartz PCI digital imaging software (Quartz Imaging Corporation, Vancouver, British Columbia, Canada). To measure valve dimensions (Hoggarth 1999) we printed photomicrographs of glochidia and measured valve dimensions to the nearest mm with a ruler. We converted measures to microns using an electronically-generated scale bar printed with each image. To measure growth of freshly-released juvenile *Q. fragosa* recovered from *Ictalurus punctatus* trials at the UMESC we used a dissecting microscope and ocular micrometer. We analyzed differences in valve dimensions among species using nested ANOVA with length or height as the dependent variable, species as the major independent factor, and individual female as the minor factor. Multiple means comparison tests (Tukey's *HSD*) were also conducted (JMP v. 5, SAS Institute, Cary, North Carolina).

Natural infestations

We collected fishes naturally infested with glochidia around mussel beds in the St. Croix River that contained *Quadrula fragosa*. We captured fishes between April to June and September to October, 1998–2005, and visually inspected them for glochidia. Infested fishes were held in flow through aquaria (40–500 L) containing well water adjusted between 11–23 °C to follow the change in St. Croix River water temperature measured at the USGS gauging station, St. Croix Falls, Wisconsin until juveniles released or glochidia were sloughed. To recover juveniles we used the procedures described above for host suitability trials.

To aid in identification of a juvenile mussel recovered from a naturally infested *Ictalurus punctatus* we also conducted host suitability trials. We exposed *I. punctatus* to glochidia from *Cumberlandia monodonta*, *Leptodea fragilis*, *Tritogonia verrucosa*, and *Truncilla truncata* during 1996–2006 using methods previously described at water temperatures between 16–20 °C. We assumed that negative results in these trials would provide additional evidence that the wild juvenile we recovered was either a *Quadrula fragosa* or *Q. quadrula*.

RESULTS

Brooding behavior

Quadrula fragosa females from the St. Croix River brooded young in all four gills during the fall. Before *Q. fragosa* began brooding some individuals, presumably females, began to emerge from the substrate as early as mid-August. We noted an increase in the incidence of emergence prior to and during the brooding period. Gravid females with swollen gills or a mantle magazine were observed once (24 September) in 1997. In 1998 we observed females brooding between 10 September to 8 October at 15–21 °C with the percentage of gravid females peaking at 58% on 24 September (Fig. 1). Between 2000–2010 we observed brooding animals between 8–29 September. The youngest and oldest gravid females we observed were 8 and 18 y, respectively.

Brooding *Quadrula fragosa* exhibited several characteristics useful to propagation efforts. Brooding females were

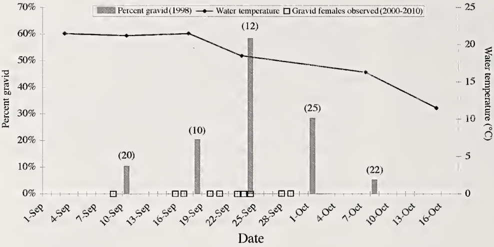

Figure 1. Brooding period of *Quadrula fragosa* in the St. Croix River during 1998. Numbers in parentheses are the number of females checked for gravidity. The black line denotes water temperatures and the unfilled squares denote dates gravid *Quadrula fragosa* were observed during 2000–2010.

relatively obvious as they often had half or more of their valves raised above the river bed, and frequently presented a swollen, gray, crenulated mantle magazine just dorsal to the incurrent aperture (Fig. 2). We usually selected females with fully developed displays when seeking mature glochidia. Since brooding females sometimes released glochidia when removed from the substrate, divers placed them in separate sealed plastic bags before bringing them onto the boat. To determine if a non-displaying mussel was brooding glochidia, we opened the valves slightly and illuminated the gills for examination. Gills bearing glochidia were only 2–3 fold thicker than non-brooding gills. Gills bearing glochidia often had fine white lines, ~1 mm wide, oriented 45–90° to gill filaments. These lines often consisted of a series of small white dots that contrasted in color with the yellowish-white gills, and were presumably conglutinates.

Gravid *Quadrula fragosa* held conglutinates and glochidia in their marsupia or within the magazine. *Quadrula fragosa* released glochidia in whole and broken conglutinates and individually in the laboratory (Fig. 3). Conglutinates were white in color, had an elongated rectangular outline that often tapered to a rounded point and some conglutinates were joined at one end. Whole conglutinates were 50–120 mm long and 2–3 mm wide.

Host suitability trials

More than 28,000 juvenile *Quadrula fragosa* were recovered during host suitability trials. Of the 67 fish species (in 20 families) and *Necturus maculosus* exposed to *Q. fragosa* glochidia, only *Ictalurus furcatus* and *I. punctatus* facilitated metamorphosis (Table 1). *Ictalurus furcatus* appeared to facilitate metamorphosis of a greater proportion of glochidia than *I. punctatus* where a similar number of glochidia were exposed to fishes. However, females contributing glochidia and glochidia exposure times varied between trials, and our small number of replicate trials prevented statistical comparison of juvenile production between fish species. *Ictalurus furcatus* maintained at 20 °C produced several thousand juveniles after 20–50 d of attachment (Table 1, trials 2–5). In contrast, *I. furcatus* maintained at 11 °C took 70–230 d to produce juveniles (Table 1, trial 1). The data for *I. punctatus* were considerably more variable, but in general, metamorphosis took longer at 11 °C than at warmer temperatures (Table 1, compare trials 7 and 18). The length of attachment periods was similar between *I. furcatus* and *I. punctatus* at 20 °C (Table 1, compare trials 2–5 to trials 14–17). We did not obtain enough glochidia to have replicate tests within a given fish source, so we cannot address this potential source of variation in our trials.

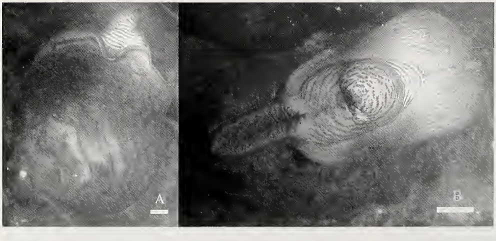

Figure 2. Image of gravid *Quadrula fragosa* in the St. Croix River on 17 September 2010; **A**, posterior view and **B**, lateral view. Scale bars = 1 cm. Photo credit to Nick Rowse, U.S. Fish and Wildlife Service.

Survival of host fish was 100% in the flow through systems used at the UMESC. No disease outbreaks occurred presumably due to higher test temperatures that facilitated metamorphosis during a brief period (~6 wk). Survival of host fish was considerably more

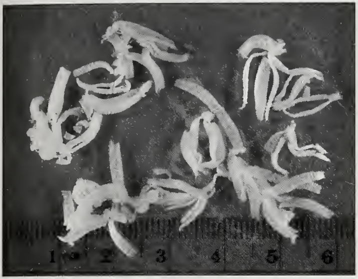

Figure 3. Conglutinates and glochidia of *Quadrula fragosa*.

variable at UMN, presumably due to *Ichthyophthirius multifilis* outbreaks among *Ictalurus punctatus* during the prolonged periods of metamorphosis at cooler temperatures. These data illustrate the importance of maintaining potential host fish health before and during testing. However, even though fish health problems were not encountered at the UMESC, the number of juveniles produced in trials using the same fish species (from the same source) and conducted at the same test temperature varied 500-fold (Table 1, trials 14–17). This variation is likely attributed to the fact that we did not quantify the number of glochidia exposed to fish and because different females were used in these trials (Table 1).

Glochidia morphology

Quadrula fragosa glochidia were readily distinguished from the glochidia of 6 other St. Croix River mussel species that also produce small glochidia (Fig. 5). Glochidial valve height and length varied significantly among species ($F_{6, 196}$ = 1660, $P < 0.0001$ and $F_{6, 198}$ = 1770, $P < 0.0001$, respectively). Glochidia height was significantly different between all species except for *Truncilla truncata* vs. *T. donaciformis*, and *T. donaciformis* vs. *Cumberlandia monodonta*, and glochidia length was significantly different between all species except for *T. truncata* and *T. donaciformis* (Table 2). In addition, glochidia from *C. monodonta* were more spherical than the other species and glochidia from *Leptodea fragilis* had a pitted surface. There were significant differences in glochidia height and length among females within species ($F_{32, 196}$ = 13.8, $P <$ 0.0001 and $F_{32, 198}$ = 7.1, $P < 0.0001$, respectively).

Quadrula fragosa grew during attachment in some trials. *Quadrula fragosa* glochidia had a subelliptical valve shape, short dorsal alae, and exterior surface sparsely-malleated dorsally. Glochidia valve height averaged 102 ± 2 µm and 86 ± 3 µm in length (n = 24, ± 1 *SD*). Newly-released juveniles (261–263 d post-exposure on *Ictalurus punctatus* at 0–21 °C) averaged 340 ± 3 µm in height and 370 ± 4 µm in length (n = 35). These changes represent about a 3-fold increase in the height and a 4-fold increase in the length of valves while attached to the host fishes (Fig. 4). *Quadrula fragosa* glochidia also increased up to two-fold in length while attached to black bullhead (*Ameiurus melas*), brown bullhead (*A. nebulosus*), yellow bullhead (*A. natalis*), *Noturus exilis*, and flathead catfish, although metamorphosis was not observed on these, or other species tested (Table 1). *Quadrula fragosa* glochidia did not increase in size during attachment to any other potential host species.

Natural infestations

We collected 689 fishes representing 43 species in 17 families from the St. Croix River and recovered 535 juvenile mussels from 15 species. Of the juveniles we recovered, only one (from an *Ictalurus punctatus*) had a glochidial valve height (101 µm), length (88 µm), and outline similar to *Quadrula fragosa*. The only mussel species whose observed range in glochidia size encompassed these measurements was *Q. fragosa*. In host suitability trials, *I. punctatus* sloughed glochidia from *Cumberlandia monodonta* (number of trials, mean number of fish per trial; 3, 4), *Leptodea fragilis* (1, 5), *Tritogonia verrucosa* (5, 5), and *Truncilla truncata* (1, 4) in < 12 d at 17 ± 3 °C, providing supporting evidence that the juvenile recovered from the naturally infested *I. punctatus* was a *Q. fragosa* or *Q. quadrula*.

DISCUSSION

The period when *Quadrula fragosa* broods its young appears to be unique. Most *Quadrula* species (10 of 13 species researched) brood glochidia between May to August although two species begin brooding in April and one has been observed brooding in January in Texas (Howard 1914, Utterback 1915, Ortmann 1919, Baker 1928, Yeager and Neves 1986, Yeager and Saylor 1995, Howells *et al.* 1996, Howells 2000, Haag and Warren 2003). To our knowledge, *Q. fragosa* is the only *Quadrula* that has been observed brooding in September, and in fact, no other Mississippi River basin mussel species has been reported brooding glochidia briefly in the fall. Thorough brooding period studies are needed for many North American freshwater mussels (Parmalee and Bogan 1998, Williams *et al.* 2008). Future studies will reveal whether or not the brief fall *Q. fragosa* brooding period is indeed truly unique.

We conducted host suitability trials on 67 potential host fishes in 20 families, representing 72% of fish species (79% of fish families) native to mid- and lower reaches of the St. Croix

Table 1. Fish species that facilitated metamorphosis of *Quadrula fragosa* glochidia into juveniles in the laboratory. Sources of host fish were: SCR-Saint Croix River, Minnesota or Wisconsin; CROW-Crow River in Minnesota; MSR-Mississippi River, Minnesota; Minnesota DNR-Minnesota broodstock, raised in a Minnesota Department of Natural Resources hatchery; and USGS-*Ictalurus punctatus* from Senecaville State Fish Hatchery, Ohio, and *I. furcatus* from Normandy Fish Hatchery, Tennessee. "Individual female(s) used for exposure" column lists identification numbers.

Trial	Host fish source	Testing facility	Individual female(s) used for exposure	Mean daily temperature[a] (°C)	Length (mm)	No. fish exposed	Estimated no. glochidia exposed to fish	No. surviving fish	Release period in days[b]	No. live juveniles recovered	Percentage of meta-morphosed glochidia
					Ictalurus furcatus						
1	USGS	UMN	3, 4, 5	11	160–200	6	[c]	2[d]	71–230	2182	[c]
2	USGS	UMESC	2	20	185–210	5	2120	5	30–49	477	19
3	USGS	UMESC	2	20	163–213	5	18960	5	27–49	3974	40
4	USGS	UMESC	5	20	168–194	5	70200	5	26–49	4283	72
5	USGS	UMESC	7	20	178–211	5	24325	5	21–49	2222	50
6	USGS	UMN	1, 2	22	180–260	7	[c]	7	28–56	813	[c]
					Ictalurus punctatus						
7	CROW	UMN	1, 2, 5	11	60–80	15	[c]	8	61–88	9	[c]
8	MSR	UMN	1, 2, 5	11	210–320	5	[c]	1	88–225	743	[c]
9	SCR	UMN	8	11	300	1	[c]	0	117–123	2	[c]
10	SCR	UMN	3	11	210–360	3	[c]	2	(33–38)	0	[c]
11	USGS	UMN	9	11	200–300	4	[c]	0	139–260	252	[c]
12	USGS	UMESC	5	[0–21]	237–263	7	> 87750	7	243–270	3450	[c]
13	USGS	UMESC	5	[13–20]	263–300	5	> 87750	5	60–89	9337	[c]
14	USGS	UMESC	5	20	137–156	5	70200	5	26–40	533	14
15	USGS	UMESC	2	20	129–143	5	2120	5	36	1	0.1
16	USGS	UMESC	2	20	125–146	5	18960	5	28–40	18	1
17	USGS	UMESC	7	20	133–161	5	24325	5	23–37	49	2
18	CROW	UMN	3	22	50–80	20	[c]	12	34–38	1	[c]
19	MN DNR	UMN	2	22	200–300	3	[c]	3	(9–14)	0	[c]
20	MSR	UMN	3	22	230–300	3	[c]	3	(34–38)	0	[c]
21	SCR	UMN	1, 2	22	300	3	[c]	1	(14–24)	0	[c]

[a] Bracketed data are mean daily temperature ranges for trials with thermal regimes that varied > 2 °C

[b] Data in parentheses are glochidia attachment periods where metamorphosis was not observed

[c] Number not estimated

[d] All 6 fish were alive up to day 216

Fish species (number of trials, mean number of fish per trial, range of days to rejection) that did not facilitate glochidia metamorphosis at 11°C unless noted otherwise (* some or all trials at 19–21°C): (1) Native fishes-*Acipenser fulvescens* (2, 3, 1–6), *Scaphirhynchus platorynchus* (1. 2, 1–2), *Lepisosteus osseus* (1, 4, 1–4)*, *Lepisosteus platostomus* (1, 4, 1–4)*, *Amia calva* (1, 2, 1–2), *Anguilla rostrata* (1, 2, [e])*, *Campostoma anomalum* (2, 8, 8–11), *Carassius auratus* (1, 1, 1–3), *Cyprinella spiloptera* (3, 9, 1–4), *Luxilus cornutus* (2, 8, 1–4), *Nocomis biguttatus* (3, 6, 1–4), *Notemigonus crysoleucas* (2, 2, 1–4)*, *Notropis atherinoides* (2, 11, 1–3), *Notropis blennius* (2, 6, 1–3), *Notropis dorsalis* (1, 22, 1–3), *Notropis hudsonius* (1, 6, 1–4), *Notropis stramineus* (2, 12, 1–4), *Phoxinus eos* (1, 19, 1–2), *Pimephales notatus* (3, 11, 1–4), *Pimephales promelas* (3, 13, 1–4), *Rhinichthys cataractae* (3, 14, 7–15), *Semotilus atromaculatus* (2, 2, 2–5), *Catostomus commersoni* (1, 14, 1–3), *Hypentelium nigricans* (2, 1, 1–4)*, *Moxostoma macrolepidotum* (1, 22, 1–3), *Ameiurus melas* (5, 11, 20–120)[f], *Ameiurus natalis* (5, 9, 8–118)[f], *Ameiurus nebulosus* (1, 4, 25–27)[f], *Noturus exilis* (8, 3, 14–95)*[f], *Noturus flavus* (2, 2, 1–6), *Noturus gyrinus* (4, 8, 4–15), *Pylodictus olivaris* (8, 3, 8–82)*[f], *Esox lucius* (1, 8, 2–8), *Umbra limi* (2, 5, 6–15), *Percopsis omiscomaycus* (1, 1, 1–4)*, *Lota lota* (3, 2, 2–11), *Fundulus diaphanus* (1, 2, 1–4)*, *Culaea inconstans* (3, 3, 22–26), *Cottus bairdii* (2, 7, 2–8), *Cottus cognatus* (1, 13, 2–8), *Morone chrysops* (1, 5, 1–4)*, *Ambloplites rupestris* (1, 17, 1–2), *Lepomis cyanellus* (3, 12, 15–21), *Lepomis gibbosus* (1, 4, 1–2), *Lepomis humilis* (2, 2, 1–4), *Lepomis macrochirus* (4, 4, 7–15), *Micropterus dolomieu* (1, 12, 1–2), *Micropterus salmoides* (2, 11, 6–15), *Pomoxis nigromaculatus* (3, 5, 6–15), *Ammocrypta clara* (1, 21, 1–3), *Crystallaria asprella* (1, 2, 1–3), *Etheostoma exile* (2, 7, 4–7), *Etheostoma caeruleum* (2, 9, 7–15), *Etheostoma flabellare* (1, 14, 1–4), *Etheostoma nigrum* (4, 16, 4–7), *Perca flavescens* (2, 28, 3–7), *Percina caprodes* (3, 8, 3–7), *Percina maculata* (1, 24, 4–7), *Percina phoxocephala* (2, 3, 2–5), *Percina shumardi* (1, 4, 1–4), *Sander canadensis* (1, 4, 1–2), *Sander vitreus* (1, 10, 1–2), *Aplodinotus grunniens* (2, 3, 4–9)*, (2) Non-native fishes-*Oncorhynchus mykiss* (1, 1, 1–4)*, *Poecilia reticulata* (2, 4, 13–15), (3) Amphibians-*Necturus maculosus* (1, 2, 1–2).

[e] Attachment period unclear

[f] Glochidia grew during some trials.

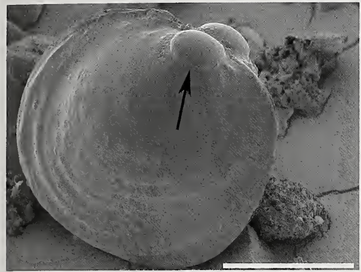

Figure 4. Newly-released juvenile *Quadrula fragosa* recovered from an *Ictalurus punctatus* held at 11°C. Note the glochidia valves (indicated by an arrow) located along the hinge of the juvenile valves. Scale bar = 200 µm.

River (Becker 1983, Fago 1986), and only *Ictalurus furcatus* and *I. punctatus* facilitated glochidia metamorphosis. This is the first time *I. furcatus* has been shown to facilitate metamorphosis of *Quadrula fragosa* under laboratory conditions. In rivers with extant *Q. fragosa* populations *I. punctatus* occurs in mid- and lower river reaches of the Bourbeuse, Kiamichi, Ouachita, St. Croix, and Saline rivers. Meanwhile, *I. furcatus* is less abundant in the downstream reaches of these rivers except for the St. Croix and Bourbeuse rivers where they do not occur (R. L. Benjamin, J. C. Boxrucker, S. E. McMurray, J. A. Olive, J. W. Quinn and D. B. Zapitello, pers. comm.). Given that *I. furcatus* has not been reported from the St. Croix River since they were stocked in 1977 (Phillips *et al.* 1982) and juvenile *Q. fragosa* have recently been found in the St. Croix River (D. J. Hornbach and M. C. Hove, pers. obs.), the absence of *I. furcatus* does not likely limit this *Q. fragosa* population. A large *I. punctatus* population does exist in the St. Croix River (Fago 1986). Future research efforts should include determining natural infestation rates of *I. furcatus* and *I. punctatus* in other rivers, comparing juvenile production rates between suitable host species, and determining host suitability of members of at least four fish families not analyzed in this study (*e.g.* Petromyzontidae, Hiodontidae, Clupeidae, Atherinopsidae).

Quadrula fragosa has among the most specific host requirements of any mussel species. A single host species has been suggested for only a few mussel species (Watters 1994), although this may be due to limited study. Many unionid species will metamorphose on several suitable hosts (cf., Watters 1994, 2010). The fact that *Ictalurus punctatus* is currently common and widely-distributed in the Mississippi River basin suggests that although *Q. fragosa* has very specific host requirements, its endangered status is probably due to factors other than limited host abundance.

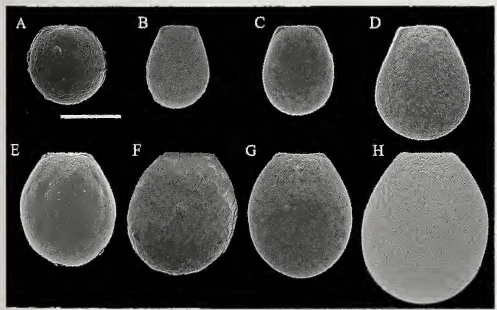

Figure 5. Scanning electron micrographs of glochidia from unionid mussels in the St. Croix River that release small glochidia (scale bar = 50 µm). Species are: A, *Cumberlandia monodonta*, B, *Truncilla donaciformis*, C, *T. truncata*, D, *Leptodea fragilis*, E, *Quadrula quadrula*, F, glochidial valve (100 µm in valve height) from a juvenile mussel recovered from a naturally infested *Ictalurus punctatus*, G, *Quadrula fragosa*, and H, *Tritogonia verrucosa*.

The rate of glochidia metamorphosis for *Quadrula fragosa* appears to be temperature-dependent, with metamorphosis occurring more rapidly at warmer temperature. This supports earlier research that showed the rate of metamorphosis for *Q. fragosa* ranged from 28–37 d in a constant (19 °C) thermal regime, totaled 70 d in a varied (12–19 °C) thermal regime, and ranged from 260–262 d in simulated natural (0–21 °C) thermal regimes (Steingraeber *et al.* 2007). These data indicate that St. Croix River *Q. fragosa* infesting a host early in the brooding period could metamorphose during a warm fall and those attaching later would likely over-winter on the host. The positive relationship between metamorphosis rate and temperature is well established in other mussel species (Roberts and Barnhart 1998, Araujo *et al.* 2002, Gray *et al.*

Table 2. Mean glochidia valve heights and lengths, standard deviations and ranges observed for St. Croix River mussel species that produce small glochidia (*i.e.* valve height < 120 μm). Valve heights and lengths with different superscripts were significantly different ($P < 0.050$).

Species	No. females	No. of glochidia measured	Mean valve height (μm ± 1 SD)	Height range	Mean valve length (μm ± 1 SD)	Length range
Cumberlandia monodonta	8	39	61 ± 3^{a}	55–67	61 ± 2^{a}	57–64
Truncilla donaciformis	3	16	$61 \pm 2^{a,b}$	56–64	47 ± 3^{b}	42–52
Truncilla truncata	4	21	66 ± 4^{b}	58–72	52 ± 3^{b}	46–56
Leptodea fragilis	6	23	89 ± 11^{c}	57–99	76 ± 6^{c}	59–82
Quadrula quadrula	2	19	92 ± 4^{d}	83–97	84 ± 3^{d}	80–91
Quadrula fragosa	5	24	102 ± 2^{e}	96–107	86 ± 3^{e}	82–92
Tritogonia verrucosa	11	57	119 ± 6^{f}	108–132	102 ± 4^{f}	92–111

2002). Even though these data show that warmer culture temperatures can produce juveniles in shorter periods of time, at lower cost, and with fewer losses of host fish, potential pitfalls warrant caution in adopting this approach to propagation. In the flat floater (*Anodonta suborbiculata* Say, 1831), a species that releases glochidia in the winter, the percentage of glochidia that metamorphosed was inversely related to water temperature (Roberts and Barnhart 1998). Juvenile *Q. fragosa* that metamorphosed over 8 to 9 months under conditions mimicking seasonal variations in the St. Croix River (0–21 °C) had valve morphology characteristic of the species, while juveniles that metamorphosed after 2 months under unseasonably warm temperatures (12–20 °C) were atypical (Steingraeber *et al.* 2007). M. C. Barnhart (pers. comm.) observed *Q. fragosa* juveniles release earlier at 23 °C but they were smaller than those held through the winter at lower temperatures. These findings indicate the need to carefully evaluate many factors that may influence glochidial metamorphosis to ensure not only high levels of viability, but also expression of natural genetic traits among propagated juveniles.

Culture of juvenile *Quadrula fragosa* should follow the natural thermal regime. It has been suggested that the parasitic stage of mussels serves three functions: (1) metamorphosis, (2) timing of excystment to minimize mortality in the first y, and (3) dispersal (Jokela *et al.* 1991). Even though *Q. fragosa* can be cultured quickly in warm water and restocked in late fall (function 1), this action may increase overwintering mortality of juveniles (function 2). Overwinter mortality may be decreased through two mechanisms. First, juveniles can be grown to a size that is less susceptible to predation in the wild (Beaty and Neves 2004). Second, the quantity and quality of food resources available during winter may not be adequate for survival. Food resources in the upper Mississippi River vary seasonally with diatoms dominating in the spring and green algae becoming more abundant during late spring (Baker and Baker 1981, Huff 1986, Luttenton *et al.* 1986). Although we know very little about the nutritional requirements of juveniles, green algae and diatoms appear to be important in their diets, at least in laboratory culture (Gatenby *et al.* 1996, 1997). In the marine mussels, the timing of larval food has been shown to be important for performance of juveniles (Phillips 2004). Thus, we suggest culturing juvenile *Q. fragosa* under a natural thermal regime until there is a greater understanding of the factors influencing juvenile survival.

Glochidia growth while encysted occurs in few mussel species. Glochidia of pimpleback (*Quadrula pustulosa pustulosa* (I. Lea, 1831)), *Q. quadrula* and *Tritogonia verrucosa* are all reported to grow while encysted on ictalurids (Howard 1913, Howard and Anson 1922, Pepi and Hove 1997). In addition to *Quadrula*, growth of encysted glochidia is reported in only two other mussel lineages: *Margaritifera* spp. (Nezlin *et al.* 1994), and the Lampsiline clade including *Potamilus* Rafinesque, 1818 and *Truncilla* Rafinesque, 1819 (Surber 1913, Utterback 1916, Coker *et al.* 1921). Perhaps selective pressures exist for juvenile mussels to reach a minimum size (e.g. 300–400 μm) or shape (e.g. discoidal) before they release from their hosts. For example, anterior-posterior valve growth closes the large gap between glochidial valves in *Potamilus* spp. thereby improving the defensive roll of the shell. Or perhaps a larger size is needed for juvenile mussels to settle in appropriate habitat after leaving their host (Barnhart *et al.* 2008). Additional work is needed to determine whether glochidial growth occurs among other mussel species, especially those that release small glochidia, and to fully elucidate the adaptive significance of this trait.

Several facts support our identification of the juvenile mussel recovered from the naturally infested *Ictalurus punctatus* as a *Quadrula fragosa*. First, glochidia valve characters were consistent with previous studies (*e.g.* Hoggarth 1999). Second, *Q. fragosa* metamorphose on *I. punctatus*, but many St. Croix River mussel species with small glochidia (*Cumberlandia monodonta*, *Leptodea fragilis*, *Tritogonia verrucosa*, and *Truncilla* *truncata*) do not (Howells 1997). Another St. Croix River species that produces small glochidia is *Q. quadrula*. This species broods glochidia during April to August (Howard 1914, Coker *et al.* 1921), and grow and metamorphose on *I. punctatus* between 51–68 d at rising water temperature from 13–20 °C (Schwebach *et al.* 2002). Juvenile *Q. quadrula* and *Q. fragosa* are probably released naturally in the St. Croix River during May and June, and we recovered the juvenile described in this study during June from a fish caught mid-May. However, the glochidial valve height of the recovered juvenile was greater than the range observed for *Q. quadrula* glochidia. Third, *Q. quadrula* are very rare in the reach of the St. Croix River where *Q. fragosa* occur. No *Q. quadrula* were observed among 4043 mussels collected during 10 y of sampling in St. Croix River's primary *Q. fragosa* bed, yet *Q. fragosa* consisted of 0.1% of the mussel community (Hornbach 2001). Collectively, these data lead us to conclude that the juvenile mussel recovered from the naturally infested *I. punctatus* was a *Q. fragosa*.

Conservation of *Quadrula fragosa* will partly depend on meeting the species' early life history needs. We have shown that *Ictalurus punctatus* and *I. furcatus* are likely hosts in the wild and can be used to successfully propagate *Q. fragosa* in captivity. Although propagation is more rapid at warmer temperatures, the thermal environment in which juveniles are restocked needs to be considered in any propagation program. It is unlikely that the decline of *Q. fragosa* is currently limited by host fish, as *I. punctatus* is relatively abundant in the St. Croix River. However, given that there is a small commercial fishery for *I. punctatus* in the St. Croix River and that *I. punctatus* probably overwinter in Lake St. Croix near the confluence with the Mississippi River where commercial fish harvest is greater (J. Gordon and D. B. Zapitello, Minnesota Department of Natural Resources (DNR), M. Marron and R. L. Benjamin, Wisconsin, DNR, pers. comm.), we suggest that resource managers determine the summer and winter ranges of *I. punctatus* in the St. Croix River, and evaluate the impact of *I. punctatus* on both glochidial mortality and availability of hosts for *Q. fragosa*.

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Genetic diversity of two common freshwater mussel species, *Lampsilis cardium* and *Quadrula pustulosa* (Bivalvia: Unionidae), in a large federally protected waterway (St. Croix River, Minnesota/Wisconsin, U.S.A.)

Suzannah C. Szumowski[1], **Sarah L. Boyer**[2], **Daniel J. Hornbach**[2], **and Mark C. Hove**[2]

[1] Division of Biological Sciences, University of California, San Diego, California 92093 U.S.A.

[2] Biology Department, Macalester College, 1600 Grand Avenue, Saint Paul, Minnesota 55105 U.S.A.

Correspondence, Sarah L. Boyer: boyer@macalester.edu

Abstract: Freshwater mussels in the family Unionidae have suffered severe population declines because of severe anthropogenic disturbances, such as habitat destruction and habitat alteration. Understanding the genetic diversity of healthy unionid populations is crucial to developing informed management plans for imperilled mussels. Here, we characterize the genetic diversity of two common species, *Lampsilis cardium* Rafinesque, 1820 and *Quadrula pustulosa* (I. Lea, 1831), using the mitochondrial gene ND1. Populations of *Q. pustulosa* contained more numerous and more highly divergent haplotypes than populations of *L. cardium*. This disparity in genetic diversity could be because of several factors, including differences in population size and location and extent of refugia during the Last Glacial Maximum. For both species, AMOVA analysis indicated no genetic structuring based on location within the river, with genetic diversity concentrated within rather than between populations. This finding is consistent with patterns seen for other common mussel species characteristic of large rivers.

Key words: aquatic, conservation genetics, population genetics, mtDNA, ND1

Mussels in the family Unionidae are important components of freshwater ecosystems with 680 species recognized worldwide (Graf and Cummings 2009). Alarmingly, the International Union for Conservation of Nature and Natural Resources Red List identifies 261 unionid species as extinct, endangered, or threatened (IUCN 2008). The overwhelming majority of these species reside in North America (Lydeard *et al.* 2004) and are in decline throughout their range (Williams *et al.* 1992), with 70 species in the United States listed as federally endangered or threatened (U.S. Fish and Wildlife Service 2009). To further understanding of unionid ecology and evolution, population genetic studies of unionids have proliferated in recent years, including some work on rare or endangered species (*e.g.*, Buhay *et al.* 2002, Machordom *et al.* 2003, Kelly and Rhymer 2005, Grobler *et al.* 2006, Zanatta and Murphy 2008, Grobler *et al.* 2011), as well as studies focused on common species (*e.g.*, Mock *et al.* 2004, Berg *et al.* 2007, Burdick and White 2007, Wolfe *et al.*, 2007, Elderkin *et al.* 2008).

The St. Croix River is a major drainage traversing some 164 miles in Minnesota and Wisconsin, U.S.A. Despite several major anthropogenic disturbances to the St. Croix, such as intensive logging in the late 1800s and the construction of a hydroelectric dam at the historic St. Croix Falls in 1903, it has remained a high-quality habitat for freshwater mussels. It was one of the original rivers included in the National Wild and Scenic River Act of 1968 and since then has been subjected to few human disturbances in comparison with other major rivers in the region, most notably the Mississippi. There are 48 species of freshwater mussels native to Minnesota, and 40 of them reside in the St. Croix (National Park Service 2008). These 40 species are, or were, also widely distributed throughout the upper Mississippi River drainage, and it is possible that the St. Croix may provide a refuge for a significant component of the upper Mississippi River mussel fauna. Unlike many large Midwestern drainages, the St. Croix River has yet to be infiltrated by zebra mussels throughout the majority of its length, with populations currently limited to the furthest downstream reach. Because of the global decline in number and diversity of unionids, the native mussels of the St. Croix River have received significant attention from local ecologists, including multiple long-term community monitoring studies (Hornbach *et al.* 1996, Hornbach 2001, National Park Service 2008). Understanding the genetic diversity and population structure of healthy and recruiting unionid populations, such as the diverse and well-studied mussel assemblage of the St. Croix, is particularly desirable as propagation and re-introduction efforts are undertaken for mussels extirpated from the Mississippi River.

The life cycle of unionid species involves a larval stage, the glochidium that parasitizes the gills or fins of fishes and is thought to function primarily to facilitate dispersal as fishes are much more vagile than mussels. Although the St. Croix River is less disturbed than other major drainages in the region, the dam at St. Croix Falls probably acts as a barrier to upstream movement of host fishes, potentially affecting mussel distribution (Kelly and Rhymer 2005, Haponski *et al.* 2007, Barnhart *et al.* 2008). Fish distribution in the St. Croix River differs up and downstream of the St. Croix Falls Dam with several fishes confined to downstream areas (*e.g.*, *Pylodictis olivaris* (Rafinesque, 1818), *Sander canadensis* (Griffith and Smith, 1834), and *Ammocrypta clara* Jordan and Meek, 1885 (Fago 1986), consistent with the idea that the dam acts as a barrier to fish dispersal. The geographic range of appropriate fish hosts is known to limit the geographic range of mussel species (Strayer 2008); therefore limits on fish movement imposed by the dam and historic falls may affect the population structure of mussels in this area. Fish host vagility is also thought to affect population structure, with more vagile hosts associated with lower levels of genetic differentiation between mussel populations (*e.g.*, Berg *et al.* 2007, Elderkin *et al.* 2008).

Previous studies have demonstrated that the mussel assemblages in areas of the St. Croix River above and below the St. Croix Falls also differ, with the subfamilies Lampsilinae (Tribe Lampsilini *sensu* Graf and Cummings 2007) and Ambleminae (Tribes Amblemini + Quadrulini + Pleurobemini *sensu* Graf and Cummings 2007) varying in prevalence above versus below the St. Croix Falls dam (Hornbach, 2001 Fig. 12.8). This variability is not likely to be a result of variation in microhabitat factors (Hornbach 2001), and it is possible that the historic falls and current dam have restricted fish movement and thereby influenced mussel community structure in the St. Croix River. Although the age of the historic falls is not known, this region of the river has been free of ice for 12,000 years (Crawford 1994) and thus a natural physical barrier to fish passage may have existed for a significant period of time prior to the construction of the St. Croix Falls dam.

In the current study, we examine the population genetic diversity and structure of two unionids common in the St. Croix River, each of which is congeneric with an endangered species from the St. Croix: *Lampsilis cardium* Rafinesque, 1820 (congeneric with *L. higginsii* (I. Lea, 1857), the endangered Higgins eye) and *Quadrula pustulosa* (I. Lea, 1831) (congeneric with *Q. fragosa* (Conrad, 1835), the critically endangered winged mapleleaf). Therefore, the data presented here can serve as a point of comparison for conservation managers interested in preserving the genetic diversity of *L. higginsii* and *Q. fragosa*. Characterization of healthy populations is crucial to ensuring that conservation decisions are made from an informed perspective with defined goals in mind. In addition, we compared the genetic diversity of the two species and performed tests for population genetic structure associated with the St. Croix Falls dam.

MATERIALS AND METHODS

Sample Collection

Samples were taken from June - July 2007 from four locations known to have large populations on the St. Croix River (Hornbach 2001); two locations above the St. Croix Falls dam (Wild River and Seven Islands) and two below (Peaslee and Hudson) (Fig. 1). GPS coordinates for each collection site were recorded and individuals were photographed for voucher purposes (Table 1). Voucher photographs for all specimens are publicly available through MorphoBank, (morphobank.org), project 376. At each location, a small amount of mantle tissue was clipped (approximately 1 cm^2) from each of 20 adult *Lampsilis cardium* and 20 adult *Quadrula pustulosa* mussels before returning the animals to the riverbed. Care was taken to avoid the reproductive areas of tissue donors, as cells from gonadal tissue have been shown to exhibit doubly parental inheritance mechanisms of mitochondrial DNA in some species of unionid mussels (Liu *et al.* 1996), which would complicate phylogenetic analyses based on mitochondrial genes. Tissue samples were preserved in 95% ethanol and later stored at -20 °C. Mussel nomenclature follows Turgeon *et al.* (1998).

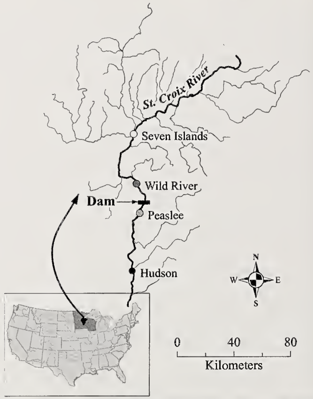

Figure 1. Tissue sample collection locations on the St. Croix River: two populations above (Seven Islands and Wild River) and two populations below (Peaslee and Hudson) the St. Croix Falls Dam were sampled; mantle tissue clippings from twenty mussels of each species, *Quadrula pustulosa* and *Lampsilis cardium*, were collected from each population.

Table 1. Tissue collection information and GenBank accession numbers. Sample ID is coded with collecting locality (H = Hudson, P = Peaslee, SI = Seven Islands, WR = Wild River), species name (C = *Lampsilis cardium*, P = *Quadrula pustulosa*), and sequential numerals. Voucher photographs for all specimens are available through MorphoBank, project 376.

	ID	**Longitude (W)**	**Latitude (N)**	**GenBank Accession #**
Lampsilis cardium	HC1–20	92°46′14.0″	44°58′31.7″	FJ601300–319
	PC1–7	92°42′08.0″	45°20′52.0″	FJ601320–26
	PC9–20	92°42′08.0″	45°20′52.0″	FJ601327–338
	PC21–22	92°42′14.7″	45°21′24.3″	FJ601339–340
	SIC1–3	92°44′41.8″	45°50′32.4″	FJ601281–83
	SIC4–12	92°49′54.0″	45°49′31.9″	FJ601284–292
	SIC14	92°49′54.0″	45°49′31.9″	FJ601293
	SIC15–20	92°49′54.0″	45°49′31.9″	FJ601294–99
	WRC1–12	92°43′44.4″	45°31′21.8″	FJ601341–352
	WRC14–17	92°43′44.4″	45°31′21.8″	FJ601353–56
	WRC19–20	92°43′44.4″	45°31′21.8″	FJ601357–58
Quadrula pustulosa	HP1–2	92°46′14.0″	44°58′31.7″	FJ601253–54
	HP4–7	92°46′14.0″	44°58′31.7″	FJ601255–58
	HP12–18	92°46′14.0″	44°58′31.7″	FJ601259–65
	HP20	92°46′14.0″	44°58′31.7″	FJ601266
	PP1–21	92°41′41.1″	45°22′01.1″	FJ601220–240
	PP22	92°42′14.0″	45°21′19.4″	FJ601241
	SIP6	92°49′54.0″	45°49′31.9″	FJ601242
	SIP8–9	92°49′54.0″	45°49′31.9″	FJ601243–44
	SIP11–14	92°49′54.0″	45°49′31.9″	FJ601245–48
	SIP17–20	92°49′54.0″	45°49′31.9″	FJ601249–252
	WRP1–2	92°43′44.4″	45°31′21.8″	FJ601267–68
	WRP11–20	92°43′44.4″	45°31′21.8″	FJ601269–278

Amplification of the ND1 Gene Region

Genomic DNA was extracted from approximately half of each mantle clipping with the DNeasy Tissue Kit (Qiagen) following the provided instructions for animal tissue extractions. Polymerase chain reaction was used to selectively amplify the NADH dehydrogenase subunit 1 (ND1) gene, which has been used in previous population studies of bivalves (Serb and Harris 2003, Serb *et al.* 2003, Campbell *et al.* 2005, Grobler *et al.* 2006, Grobler *et al.* 2011). We used the following primers: forward: 5′-TGG CAG AAA AGT GCA TCA GAT TAA AGC-3′ and reverse: 5′-CCT GCT TGG AAG GCA AGT GTA CT-3′ (Serb *et al.* 2003). To amplify ND1, 1X PCR buffer, 0.25 μM of each primer, 200 μM dNTPs, 1.25 units of *Taq* polymerase, and 50 ng of template DNA were combined in a 25 μl reaction. PCR reactions were performed in an ABI 2720 thermal cycler using the following profile: 98 °C denaturation for 2 min; 30 cycles of 10 s denaturation at 98 °C, 5 s annealing at 57 °C, and 1 min 15 s extension at 72 °C; final extension for 2 min at 72 °C. DNA was purified with a QIAquick PCR Purification Kit (Qiagen) and stored at -20 °C. A NanoDrop® ND-1000 Spectrophotometer was used to quantify the amplified DNA. Typical DNA concentrations ranged from approximately 30–40 ng/μl.

Cycle Sequencing

Cycle sequencing reactions consisted of 30–40 ng cleaned PCR product, 100 nM forward or reverse ND1 primer, and 8 μl DTCS Quickstart (Beckman Coulter) in a 20 μl reaction. DNA and primer were preheated in thermal cycler at 95 °C for 5 min before adding DTCS Quickstart. We used the following sequencing reaction program: 30 cycles of 96 °C denaturing for 20 sec, 50 °C annealing for 20 sec, and 60 °C extension for 4 min, and a final hold at 4 °C. A magnetic plate and magnetic beads (Beckman Coulter) were used with 3 washes of 85% ethanol to purify reactions. Automated sequencing was performed using a Beckman Coulter CEQ8000 sequencer with the LFR-1 separation method. Sequences were published on GenBank under the accession numbers listed in Table 1.

Data Preparation and Population Genetic Analyses

Sequence data from Beckman Coulter's CEQ8000 Genetic Analysis System v. 9.0 were edited and assembled into contigs (consensus sequences) with Sequencher (version 4.8, Gene Codes Corporation). Alignments were created using MacClade (Maddison and Maddison 2001).

Population genetic analyses were performed using Arlequin version 3.01 (Excoffier *et al.* 2005). Standard diversity indices, including number of haplotypes (*Nh*), number of polymorphisms (*Np*), haplotypic diversity (*h*), and nucleotide diversity (π_n) were calculated to assess diversity within each population. A comparison of haplotypic diversity between the two species was performed using the Wilcoxon t-test as implemented in JMP (version 7, SAS Institute Inc.). An analysis of molecular variance (AMOVA, Excoffier *et al.* 1992) was performed separately for each species to test hierarchical models of genetic variance using pairwise differences as a measure of divergence (1000 permutations). The data were partitioned as follows: individuals from each locality were considered a population, and localities above versus below the dam were considered groups. An AMOVA was run to test for differentiation between populations and between groups.

Minimum spanning networks were created using statistical parsimony in TCS 1.21 (Clement *et al.* 2000). Reticulations within the haplotype networks were resolved using the principles of coalescent theory, as described by Crandall *et al.* (1994), with interior and frequently occurring haplotypes inferred to be more probable precursors to new haplotypes.

To further compare levels of genetic diversity in the two species, mean p-distances within species, within populations (*i.e.* sampling localities in Figure 1), and between populations were calculated using pairwise deletion, and standard error was assessed for each using 500 bootstrap replicates, with MEGA version 4.0 (Tamura *et al.* 2007).

Phylogenetic Analysis

We performed separate phylogenetic analyses of the two datasets. For phylogenetic analysis of the *Lampsilis cardium* dataset, we included outgroups from across the Lampsilini (*sensu* Graf and Cummings 2007) as well as one published sequence from *L. cardium*, from the Middle Maitland River (Ontario, Canada). For phylogenetic analysis of the *Quadrula pustulosa* dataset, we included outgroups from across the Quadrulini (*sensu* Graf and Cummings 2007) as well as 13 published sequences from *Q. pustulosa* from localities across the species' range (Table 2).

The model of sequence evolution was chosen separately for each dataset using ModelTest 2.3 (Posada and Crandall 1998, Nylander 2004) using the Akaike information criterion as recommended by Posada and Buckley (2004); in each case GTR+I+Γ was selected. Phylogenetic analyses were performed separately for each dataset using MrBayes 3.1 (Huelsenbeck and Ronquist 2001) under the GTR+I+Γ model. The *Quadrula pustulosa* analysis consisted of 437,900 replicates with the first 90,000 discarded as burnin. *Lampsilis cardium* Bayesian analysis included 376,500 replicates with the first 40,000 discarded as burnin. Parsimony analyses were performed in PAUP* v 4.0 (Swofford 2002). Support was assessed with 1000 bootstrap replicates. Trees were rooted based on the findings of Campbell *et al.* (2005).

Long-Term Monitoring

Mussel communities at each of our locations of interest have been monitored at 2–5 year intervals since 1992. Community analysis was undertaken by collecting 100 x ¼ m^2 quadrats at each locality as described by Hornbach *et al.* (1996). Data were collected at Hudson during 4 seasons (400 samples total), Peaslee during 5 seasons (500 samples total), Seven Islands during 2 seasons (200 samples total), and Wild River during 6 seasons (600 samples total) for a total of 1700 samples overall.

RESULTS

Samples from 78 *Lampsilis cardium* were successfully sequenced for 869 bp of the ND1 gene. There were 34 polymorphic sites, comprising 4% of the positions sequenced (Table 2). From these 78 individuals, 22 haplotypes were identified, four of which were found in all four populations and 13 (59%) of which were unique (Table 3). The average number of individuals per haplotype was 3.5, with 50% of all individuals concentrated into the two most frequent haplotypes.

Samples from 59 *Quadrula pustulosa* were successfully sequenced to 686 bp of the ND1 gene. There were 72 polymorphic sites, comprising 11% of the positions sequenced (Table 2). From these 59 individuals, 41 distinct haplotypes were identified (Table 3). None of these 41 haplotypes was found in all four populations sampled and 35 haplotypes (85%) were unique, represented by only one individual. The average number of individuals per haplotype was 1.4, with 50% of all individuals concentrated into the 12 most frequent haplotypes.

Wilcoxon *t*-test indicated that haplotypic diversity was significantly different between the two species ($P < 0.05$ for both 2-sample test with normal approximation and 1-way test with chi-square approximation). The differences in genetic diversity of *Lampsilis cardium* relative to *Quadrula pustulosa* are represented in the minimum spanning haplotype networks generated using the program TCS (Figs. 2 and 3). The *L. cardium* dataset included 19 fewer haplotypes than the *Q. pustulosa* dataset, despite a larger sample size. Branch-lengths, which represent the number of mutations between haplotypes, are shorter in the *L. cardium* network

Table 2. Genetic diversity indices for *Lampsilis cardium* and *Quadrula pustulosa* at each sampling location. n = sample size, Nh = number of haplotypes ± *SE*, Np = number of polymorphic sites ± *SE*, h = haplotypic diversity, π_n = nucleotide diversity.

Population	Species	n	Nh	Np	h	π_n
Hudson	*L. cardium*	20	10	22	0.878 ± 0.0479	0.00584 ± 0.00330
Peaslee	*L. cardium*	21	11	21	0.900 ± 0.0472	0.00628 ± 0.00352
Seven Islands	*L. cardium*	19	8	13	0.853 ± 0.0537	0.00518 ± 0.00302
Wild River	*L. cardium*	18	9	12	0.823 ± 0.0752	0.00600 ± 0.00362
Hudson	*Q. pustulosa*	14	13	22	0.989 ± 0.0314	0.00574 ± 0.00344
Peaslee	*Q. pustulosa*	22	19	45	0.974 ± 0.0276	0.01089 ± 0.00590
Seven Islands	*Q. pustulosa*	11	8	27	0.927 ± 0.0665	0.01002 ± 0.00576
Wild River	*Q. pustulosa*	12	10	22	0.954 ± 0.0569	0.00744 ± 0.00438

compared to the *Q. pustulosa* network. Mean p-distances were smaller in *L. cardium* than *Q. pustulosa*, with values of 0.006 (*SE* = 0.001) and 0.009 (*SE* = 0.002) respectively. Mean p-distances within and between followed this trend, and for each species within-population and between-population distances were equivalent in magnitude. Within populations, mean *p*-distance ranged from 0.005–0.006 (*SE* = 0.002 in each case) in *L. cardium* and from 0.007–0.011 (*SE* = 0.001–0.002) in *Q. pustulosa*. Between populations, mean p-distance ranged from 0.005–0.006 (*SE* = 0.001–0.002) in *L. cardium* and from 0.007–0.011 (*SE* = 0.001–0.002) in *Q. pustulosa*.

No geographic structuring was observed in the minimum spanning networks. Analysis of molecular variation (AMOVA) confirmed that all variation occurred within populations and no variation occurred either among groups (above versus below the dam) or among populations (collecting localities) (Table 4).

Phylogenetic analysis of the Lampsilini data indicated that all *Lampsilis cardium* individuals, including one published sequence from the Middle Maitland River in Ontario (Canada) form a well-supported clade. Within *L. cardium* no structure related to geography is evident (Fig. 4). Phylogenetic analysis of the Quadrulini data results in *Quadrula pustulosa* rendered paraphyletic with respect to *Quadrula aurea* (I. Lea, 1859) (Fig. 5). As with *L. cardium*, we found no resolution within the St. Croix River among different sampling locations. *Q. pustulosa* sequences downloaded from GenBank (Appendix 1) representing collections from Arkansas, Illinois, Kentucky, Louisiana, Missouri, Tennessee, Virginia, and Wisconsin are interspersed with samples from the St. Croix River (Fig. 5).

Long-term mussel community monitoring data are presented in Table 5. With the exception of the Seven Islands mussel assemblage, *Lampsilis cardium* was outnumbered by *Quadrula pustulosa* at every site monitored from 1992–2009, comprising on average 2.43 ± 0.02% of the total assemblage compared to 4.05 ± 0.03% for *Q. pustulosa*.

DISCUSSION

Berg *et al.* (2007) hypothesized that mussel species characteristic of large river systems exhibit large amounts of within-population genetic variation, while species that inhabit small streams are characterized by higher between-population variation. The results of our study of two abundant species within a large river system are consistent with this hypothesis. Recent analysis of parentage of *Lampsilis cardium* individuals from Ohio waterways indicates the possibility of dispersal of spermatozoa over many kilometers in this species (Ferguson 2009), providing a potential explanation for the lack of geographic structure seen in this species and other mussels in large rivers (*e.g.*, Berg *et al.* 1998, Elderkin *et al.* 2007).

In addition to finding more within-population than between-population variation in both our species of interest, we found differences in the absolute amount of variation in the ND1 gene exhibited by *Lampsilis cardium* and *Quadrula pustulosa*, with *Q. pustulosa* displaying greater variation than *L. cardium* in terms of number of haplotypes, number of polymorphic sites, haplotypic divergence, nucleotide diversity and mean p-distances within species, within populations, and between populations (Table 2, Figs. 2 and 3). We performed a Wilcoxon *t*-test to formally compare the number of haplotypes found in populations of the two species, and this test confirmed that differences were significant. Differing levels of genetic diversity could reflect differences in population size; in three of the four sampling localities in this study, *Q. pustulosa* has larger population sizes than does *L. cardium* (Table 5). Data from 17 years of monitoring the St. Croix River mussel communities show that *Q. pustulosa* makes up on average 4.05 ± 0.03% of the total mussel assemblages at the four sampling locations, whereas *L. cardium* comprises approximately 2.43 ± 0.02% of the total assemblage (n = 1700 collected quantitatively since 1992) (Table 4). As genetic diversity is correlated with population

Table 3. Location and frequency of *Lampsilis cardium and Quadrula pustulosa* haplotypes from above and below the St. Croix Falls dam. *Lampsilis cardium* haplotypes begin with L and *Quadrula pustulosa* haplotypes begin with Q. SI = Seven Islands, WR = Wild River, P = Peaslee, H = Hudson (See map in Fig. 1).

Lampsilis cardium

	Localities			
	above		below	
Haplotypes	SI	WR	P	H
L1	2	1	2	1
L2	6	7	6	5
L3	4	4	1	5
L4	3	1	3	3
L5			2	1
L6		1	1	
L7	1			1
L9			2	
L10		1		
L11			1	
L12			1	
L13			1	1
L14	1			
L15		1		
L16	1			
L17		1		
L18		1		
L19	1			
L20				1
L21				1
L22			1	
L23				1

Quadrula pustulosa

	Localities			
	above		below	
Haplotypes	SI	WR	P	H
Q1	3	3	4	
Q2	1		1	1
Q3	2			1
Q4	1	1		1
Q5				1
Q6				1
Q7				1
Q8				1
Q9		1		2
Q10			1	
Q11			1	
Q12			1	
Q13			1	
Q14			1	
Q15			1	
Q16			1	
Q17	1		1	
Q18			1	
Q19			1	
Q20	1			
Q21	1			
Q22		1		
Q23				1
Q26			1	
Q27				1
Q28		1		
Q29		1		
Q30				1
Q31				1
Q32				1
Q33			1	
Q34			1	
Q35			1	
Q36			1	
Q37			1	
Q38			1	
Q39		1		
Q40		1		
Q41		1		
Q42	1			
Q43		1		

size, the differences in population size could explain the higher genetic diversity in *Q. pustulosa* populations. However, other hypotheses could also explain this difference. For example, it is possible that the diversity of host fishes used by each species could contribute to differing amounts of genetic variation. In addition, the number and extent of refugia

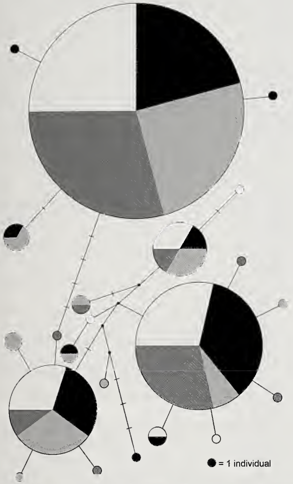

Figure 2. Minimum spanning network for *Lampsilis cardium* haplotypes based on TCS analysis: haplotype frequency is shown by proportionally scaled pie graphs, as indicated by key. Above dam locations (Seven Islands and Wild River) are shown in white and dark gray, respectively; locations below the dam (Peaslee and Hudson) are shown in light gray and black, respectively. This shading scheme matches that of Fig. 1. Hash marks on lines connecting haplotypes indicate the number of base pair changes between haplotypes, and black dots at line junctions represent unobserved haplotypes.

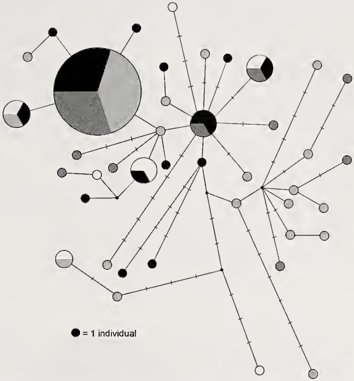

Figure 3. Minimum spanning network for *Quadrula pustulosa* haplotypes based on TCS analysis: haplotype frequency is shown by proportionally scaled pie graphs, as indicated by key. Above dam locations (Seven Islands and Wild River) are shown in white and dark gray, respectively; locations below the dam (Peaslee and Hudson) are shown in light gray and black, respectively. This shading scheme matches that of Fig. 1. Hash marks on lines connecting haplotypes indicate the number of base pair changes between haplotypes, and black dots at line junctions represent unobserved haplotypes.

occupied by these species during the Last Glacial Maximum might have been different, and could also account for the contrast in genetic diversity. It is likely a combination of the aforementioned factors that account for the observed disparities in genetic diversity between the two species.

In addition to generating data on genetic variation in healthy unionid populations within a large river, a secondary aim of this study was to determine whether the former St. Croix Falls and the 18 m high dam currently built on its location have acted as a barrier to host fish movement and thus mussel dispersal. Watters (1996) examined the impact of dams ranging in size from 1 to 17.7 m on the distribution of two species of freshwater mussels and found that animals were confined to the river sectors downstream of dams, suggesting that unionid distribution can be strongly influenced by dams. A recent study on genetic divergence in *Percina caprodes* (Rafinesque, 1818) and *Etheostoma blennioides* Rafinesque, 1819 corroborated this finding and demonstrated that dams as small as 4 m may act as unidirectional barriers to gene flow in some species of fishes (Haponski *et al.* 2007). In the present study, AMOVA revealed no structuring based on geographic distance along the river was observed, and, in fact, in both *Lampsili cardium* and *Quadrula pustulosa* several haplotypes were found at all four localities, implying that the dam and St. Croix Falls have not served as a barrier to gene flow that could be detected with the ND1 locus (Table 3).

Phylogenetic analysis of our datasets confirmed the lack of geographic structure in each species (Figures 4 and 5). Although unionid taxonomy is outside the scope of the current study, it is intriguing to note that *Quadrula pustulosa* is paraphyletic with respect to Q. *aurea* in our analyses (Figure 5), confirming the findings of Serb *et al.* (2003). *Quadrula*

Table 4. Analysis of Molecular Variance (AMOVA) indicates no genetic structuring among groups (above and below the dam), or among populations (sampling locations); variation is concentrated within populations.

Lampsilis cardium

Source of Variation	d.f.	Sum of squares	Variance components	% Variation	Fixation indices
Among groups	1	1.352	0.00516 Va	0.22	$F_{SC} = -0.02788$
Among populations within groups	2	2.299	-0.06614 Vb	-2.78	$F_{ST} = -0.02565$
Within populations	74	180.426	2.43819 Vc	102.57	$F_{CT} = 0.00217$
Total	77	184.077	2.37722		

Quadrula pustulosa

Source of Variation	d.f.	Sum of squares	Variance components	% Variation	Fixation indices
Among groups	1	2.881	-0.00586 Va	-0.19	$F_{SC} = -0.00077$
Among populations within groups	2	6.092	-0.00237 Vb	-2.78	$F_{ST} = -0.00268$
Within populations	55	169.399	3.07999 Vc	100.27	$F_{CT} = -0.00191$
Total	58	178.373	3.07176		

aurea, commonly known as the golden orb, is a critically endangered species endemic to Texas. *Quadrula aurea* and *Q. pustulosa* differ in color and presence of pustules, both of which are highly variable characters within *Q. pustulosa*. *Quadrula pustulosa* does occur in eastern Texas, and it is possible that these two morphs are not in fact reproductively isolated species, and/or have diverged very recently. A more detailed study of morphological and genetic characteristics of these two taxa could clarify their relationship.

Although it has been used in many previous population genetic studies of unionids, ND1 may be too slow-evolving to be informative about population structure associated with the construction of the dam at St. Croix Falls. Considering a longer timescale, this site has been free of glacial ice for 12,000 years (Crawford 1994), and the historic falls that existed at the dam location may therefore have acted as a barrier to fish dispersal long before the dam was constructed; however, this is not detected with the data presented here. Analysis of nuclear microsatellite loci has been used to detect population variation within unionid species that was not discernable with mitochondrial DNA (Zannatta and Murphy 2008); therefore, microsatellites may have the potential to resolve population structure related to very recent events such as the construction of the St. Croix Falls dam. However, Grobler *et al.* (2011) found genetic structure in the endangered unionid *Cyprogenia stegaria* (Rafinesque, 1820) that was detectable with the ND1 locus but not with microsatellite data. In the case of one of our species of interest, *Quadrula pustulosa*, preliminary microsatellite data have been generated, from the same set of individuals examined in the current study, for 20 loci developed to study the genetic structure of the endangered species *Q. fragosa* (Hemmingsen *et al.* 2009). These preliminary data, which are part of an unpublished study comparing genetic diversity of a common mussel (*Q. pustulosa*) and an endangered mussel (*Q. fragosa*), confirm a lack of structure associated with the St. Croix Falls dam within *Q. pustulosa* (K. Roe, unpubl. data).

The homogeneity of the genetic pool across the gradient of the St. Croix River suggests that in freshwater mussels it is normal for a high proportion of genetic diversity to occur within populations rather than between populations. Although it is important to keep in mind that our data are drawn from a single locus, this finding is consistent with the hypothesis of Berg *et al.* (2007), and indicates that conservation efforts for other *Lampsilis* and *Quadrula* species should focus not only on maintaining a large number of mussel populations per species, but also on sustaining large population sizes.

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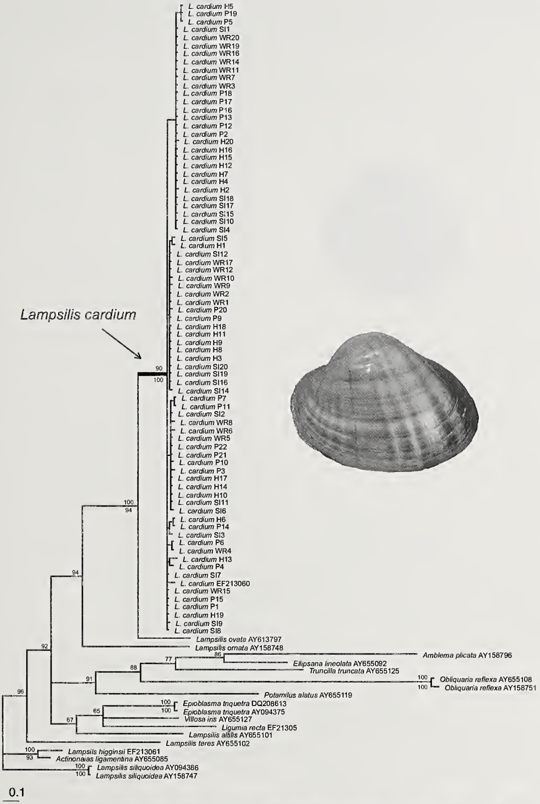
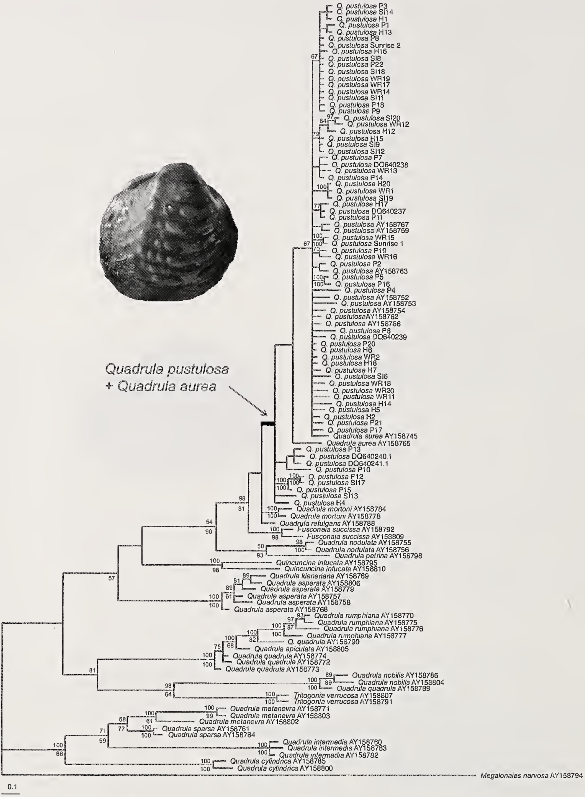

L. cardium H5
L. cardium P19
L. cardium P5
L. cardium SI1
L. cardium WR20
L. cardium WR19
L. cardium WR16
L. cardium WR14
L. cardium WR11
L. cardium WR7
L. cardium WR3
L. cardium P18
L. cardium P17
L. cardium P16
L. cardium P13
L. cardium P12
L. cardium P2
L. cardium H20
L. cardium H16
L. cardium H15
L. cardium H12
L. cardium H7
L. cardium H4
L. cardium H2
L. cardium SI18
L. cardium SI17
L. cardium SI15
L. cardium SI10
L. cardium SI4
L. cardium SI5
L. cardium H1
L. cardium SI12
L. cardium WR17
L. cardium WR12
L. cardium WR10
L. cardium WR9
L. cardium WR2
L. cardium WR1
L. cardium P20
Lampsilis cardium
L. cardium P9
L. cardium H18
L. cardium H11
L. cardium H9
L. cardium H8
L. cardium H3
90
100
L. cardium SI20
L. cardium SI19
L. cardium SI16
L. cardium SI14
L. cardium P7
L. cardium P11
L. cardium SI2
L. cardium WR8
L. cardium WR6
L. cardium WR5
L. cardium P22
L. cardium P21
L. cardium P10
L. cardium P3
L. cardium H17
L. cardium H14
L. cardium H10
100
94
L. cardium SI11
L. cardium SI6
L. cardium H6
L. cardium P14
L. cardium SI3
L. cardium P6
L. cardium WR4
L. cardium H13
L. cardium P4
94
L. cardium SI7
L. cardium EF213060
L. cardium WR15
L. cardium P15
L. cardium P1
L. cardium H19
L. cardium SI9
L. cardium SI8
Lampsilis ovata AY613797
92
Lampsilis ornata AY158748
86
Amblema plicata AY158796
77
Ellipsana lineolata AY655092
88
Truncilla truncata AY655125
91
100
Obliquaria reflexa AY655108
100
Obliquaria reflexa AY158751
96
Potamilus alatus AY655119
100
Epioblasma triquetra DQ208613
65
100
Epioblasma triquetra AY094375
Villosa iris AY655127
67
Ligumia recta EF21305
Lampsilis altilis AY655101
Lampsilis teres AY655102
100
Lampsils higginsii EF213061
93
Actinonaias ligamentina AY655085
100
Lampsilis siliquoidea AY094386
100
Lampsilis siliquoidea AY158747
0.1

Figure 4. Phylogenetic relationships of *Lampsilis cardium*. Bayesian analysis was conducted with the GTR+I+ Γ model. Numbers on branches indicate posterior probabilities (above) and parsimony bootstrap values (below) when above 50%. Support values on short internal branches within *L. cardium* are not shown because of space constraints. The thickened branch supports the monophyly of *L. cardium*. Individuals from the St. Croix River are labelled with their collecting locality (SI, WR, P, and H) and sample number. Published outgroup sequences and *L. cardium* from outside the St. Croix River are labelled with GenBank accession numbers. See Appendix 1 for more information on published data from GenBank. The shell image represents one of our voucher photographs, for individual PC14.


Q. pustulosa P3
Q. pustulosa SI14
Q. pustulosa H1
Q. pustulosa P1
Q. pustulosa H13
Q. pustulosa P8
Q. pustulosa Sunrise 2
Q. pustulosa H16
Q. pustulosa SI8
Q. pustulosa P22
Q. pustulosa SI18
Q. pustulosa WR19
Q. pustulosa WR17
Q. pustulosa WR14
Q. pustulosa SI11
Q. pustulosa P18
Q. pustulosa P9
Q. pustulosa SI20
Q. pustulosa WR12
Q. pustulosa H12
Q. pustulosa H15
Q. pustulosa SI9
Q. pustulosa SI12
Q. pustulosa P7
Q. pustulosa DQ640238
Q. pustulosa WR13
Q. pustulosa P14
Q. pustulosa H20
Q. pustulosa WR1
Q. pustulosa SI19
Q. pustulosa H17
Q. pustulosa DQ640237
Q. pustulosa P11
Q. pustulosa AY158767
Q. pustulosa AY158759
Q. pustulosa WR15
Q. pustulosa Sunrise 1
Q. pustulosa P19
Q. pustulosa WR16
Q. pustulosa P2
Q. pustulosa AY158763
Q. pustulosa P5
Q. pustulosa P16
Q. pustulosa P4
Q. pustulosa AY158752
Q. pustulosa AY158753
Q. pustulosa AY158754
Q. pustulosaAY158762
Q. pustulosa AY158766
Q. pustulosa P6
Q. pustulosa DQ640239
Q. pustulosa P20
Q. pustulosa H6
Q. pustulosa WR2
Q. pustulosa H18
Q. pustulosa H7
Q. pustulosa SI6
Q. pustulosa WR18
Q. pustulosa WR20
Q. pustulosa WR11
Q. pustulosa H14
Q. pustulosa H5
Q. pustulosa H2
Q. pustulosa P21
Q. pustulosa P17
Quadrula aurea AY158745
Quadrula aurea AY158765
Quadrula pustulosa + Quadrula aurea
Q. pustulosa P13
Q. pustulosa DQ640240.1
Q. pustulosa DQ640241.1
Q. pustulosa P10
Q. pustulosa P12
Q. pustulosa SI17
Q. pustulosa P15
Q. pustulosa SI13
Q. pustulosa H4
Quadrula mortoni AY158784
Quadrula mortoni AY158778
Quadrula refulgans AY158788
Fusconaia succissa AY158792
Fusconaia succissa AY158809
Quadrula nodulata AY158755
Quadrula nodulata AY158756
Quadrula petrina AY158798
Quincuncina infucata AY158795
Quincuncina infucata AY158810
Quadrula kieneriana AY158769
Quadrula asperata AY158806
Quadrula asperata AY158779
Quadrula asperata AY158757
Quadrula asperata AY158758
Quadrula asperata AY158768
Quadrula rumphiana AY158770
Quadrula rumphiana AY158775
Quadrula rumphiana AY158776
Quadrula rumphiana AY158777
Q. quadrula AY158790
Quadrula apiculata AY158805
Quadrula quadrula AY158774
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Quadrula quadrula AY158773
Quadrula nobilis AY158786
Quadrula nobilis AY158804
Quadrula quadrula AY158789
Tritogonia verrucosa AY158807
Tritogonia verrucosa AY158791
Quadrula matanevra AY158771
Quadrula metanevra AY158803
Quadrula metanevra AY158802
Quadrula sparsa AY158761
Quadrula sparsa AY158784
Quadrula intermedia AY158760
Quadrula intermedia AY158783
Quadrula intermedia AY158782
Quadrula cylindrica AY158785
Quadrula cylindrica AY158800
Megalonaies narvosa AY158794
0.1

Figure 5. Phylogenetic relationships of *Quadrula pustulosa*. Bayesian analysis was conducted with the GTR+I+ Γ model. Numbers on branches indicate posterior probabilities (above) and parsimony bootstrap values (below) when above 50%. Stars are used to indicate 100% when space is limited. The thickened branch indicates the most restricted clade including all *Q. pustulosa* individuals. Individuals from the St. Croix River are labelled with their collecting locality (SI, WR, P, and H) and sample number. Published outgroup samples are labelled with the species name and GenBank accession number. See Appendix 1 for more information on published data from GenBank. The shell image represents one of our voucher photographs, for individual HP1.

Table 5. Census data from long-term monitoring of collection localities. From 1992 to 2009, 1700 mussels have been surveyed at Hudson, Peaslee, Seven Islands and Wild River. The contribution of each species to the total mussel assemblage at each locality is shown as % of assemblage. Total values indicate the average percent of the assemblage for each species over all four sampling areas

		% of Assemblage		**Mussels/m^2**	
Locality	**Sample size**	*L. cardium*	*Q. pustulosa*	*L. cardium*	*Q. pustulosa*
Hudson	400	0.8%	3.7%	0.11 ± 0.06	0.53 ± 0.1
Peaslee	500	0.7%	3.5%	0.06 ± 0.06	0.26 ± 0.09
Seven Islands	200	6.0%	1.5%	0.48 ± 0.09	0.12 ± 0.14
Wild River	600	2.2%	7.5%	0.55 ± 0.05	1.90 ± 0.08
Total	1700	2.43 ± 0.02%	4.05 ± 0.03%		

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Appendix 1. GenBank accession numbers, geographic information, and authors for published sequence data incorporated in phylogenetic analyses. All samples are from either the United States or Ontario, Canada. (R = river, FMCC = Freshwater Mollusk Conservation Center).

Species	GenBank Accession #	River	State or Province	Citation
Actinonaias ligamentina (Lamarck, 1819)	AY655085	Kankakee R.	Illinois	Campbell *et al.*, 2005
Amblema plicata (Say, 1817)	AY158796	Ohio R.	Kentucky	Serb *et al.*, 2003
Ellipsaria lineolata (Rafinesque, 1820)	AY655092	Cahaba R.	Alabama	Campbell *et al.*, 2005
Epioblasma triquetra (Rafinesque, 1820)	AY094375	Clinch R.	Tennessee	Buhay *et al.* 2002
Epioblasma triquetra	DQ208613	Clinch R.	Tennessee	Jones *et al.* 2006
Fusconaia succissa (I. Lea, 1852)	AY158792	Conecuh R.	Alabama	Serb *et al.*, 2003
Fusconaia succissa	AY158809	Pea R.	Alabama	Serb *et al.*, 2003
Lampsilis altilis (Conrad, 1834)	AY655101	Etowah R.	Alabama	Campbell *et al.*, 2005
Lampsilis cardium	EF213060	Middle Maitland R.	Ontario	Zanatta and Murphy
Lampsilis higginsii	EF213061	St. Croix R.	Wisconsin	Zanatta and Murphy
Lampsilis ornata (Conrad, 1835)	AY158748	Cahaba R.	Alabama	Serb *et al.*, 2003
Lampsilis ovata (Say, 1817)	AY613797	Clinch R.	Alabama	Campbell *et al.*, 2005
Lampsilis siliquoidea (Barnes, 1823)	AY094386	Douglas Lake	Michigan	Buhay *et al.* 2002
Lampsilis siliquoidea	AY158747	Douglas Lake	Michigan	Serb *et al.*, 2003
Lampsilis teres (Rafinesque, 1820)	AY655102	Tennessee R.	Alabama	Campbell *et al.*, 2005
Ligumia recta (Lamarck, 1819)	EF213055	Sydenham R.	Ontario	Zanatta and Murphy
Megalonaias nervosa (Rafinesque, 1820)	AY158794	Coosa R.	Alabama	Serb *et al.*, 2003
Obliquaria reflexa Rafinesque, 1820	AY158751	Cahaba R.	Alabama	Serb *et al.*, 2003
Obliquaria reflexa	AY655108	Coosa R.	Alabama	Campbell *et al.*, 2005
Potamilus alatus (Say, 1817)	AY655119	Tennessee R.	Alabama	Campbell *et al.*, 2005
Quadrula apiculata (Say, 1829)	AY158805	Neches R.	Texas	Serb *et al.*, 2003
Quadrula asperata (I. Lea, 1861)	AY158757	Alabama R.	Alabama	Serb *et al.*, 2003
Quadrula asperata	AY158758	Alabama R.	Alabama	Serb *et al.*, 2003
Quadrula asperata	AY158768	Sucarnoochie Ck.	Mississippi	Serb *et al.*, 2003
Quadrula asperata	AY158779	Coosawattee R.	Georgia	Serb *et al.*, 2003
Quadrula asperata	AY158806	Coosa R.	Alabama	Serb *et al.*, 2003
Quadrula aurea	AY158745	Lake Corpus Christi	Texas	Serb *et al.*, 2003
Quadrula aurea	AY158765	Lake Corpus Christi	Texas	Serb *et al.*, 2003
Quadrula cylindrica cylindrica (Say, 1817)	AY158785	Duck R.	Tennessee	Serb *et al.*, 2003
Quadrula cylindrica strigillata	AY158800	Clinch R.	Tennessee	Serb *et al.*, 2003
Quadrula intermedia (Conrad, 1836)	AY158760	Powell R.	Virginia	Serb *et al.*, 2003
Quadrula intermedia	AY158782	Duck R.	Tennessee	Serb *et al.*, 2003
Quadrula intermedia	AY158783	Duck R.	Tennessee	Serb *et al.*, 2003
Quadrula kieneriana Lea, 1852	AY158769	Coosawattee R.	Georgia	Serb *et al.*, 2003

Appendix 1. (Continued)

Species	GenBank Accession #	River	State or Province	Citation
Quadrula metanevra (Rafinesque, 1820)	AY158771	Elk R.	Alabama	Serb *et al.*, 2003
Quadrula metanevra	AY158802	Cahaba R.	Alabama	Serb *et al.*, 2003
Quadrula metanevra	AY158803	Tennessee R.	Tennessee	Serb *et al.*, 2003
Quadrula pustulosa mortoni (Conrad, 1835)	AY158764	Big Cypress Bayou	Texas	Serb *et al.*, 2003
Quadrula pustulosa mortoni	AY158778	Lake Lewisville	Texas	Serb *et al.*, 2003
Quadrula nobilis (Conrad, 1854)	AY158786	Pascagoula R.	Mississippi	Serb *et al.*, 2003
Quadrula nobilis	AY158804	Neches R.	Texas	Serb *et al.*, 2003
Quadrula nodulata (Rafinesque, 1820)	AY158755	Neches R.	Texas	Serb *et al.*, 2003
Quadrula nodulata	AY158756	Mississippi R.	Missouri	Serb *et al.*, 2003
Quadrula petrina (Gould, 1855)	AY158798	Concho R.	Texas	Serb *et al.*, 2003
Quadrula pustulosa	AY158752	Ouachita R.	Arkansas	Serb *et al.*, 2003
Quadrula pustulosa	AY158753	Ouachita R.	Arkansas	Serb *et al.*, 2003
Quadrula pustulosa	AY158754	Mississippi R.	Missouri	Serb *et al.*, 2003
Quadrula pustulosa	AY158759	St. Croix R.	Wisconsin	Serb *et al.*, 2003
Quadrula pustulosa	AY158762	Wolf R.	Tennessee	Serb *et al.*, 2003
Quadrula pustulosa	AY158763	Ohio R.	Kentucky	Serb *et al.*, 2003
Quadrula pustulosa	AY158766	Amite R.	Louisiana	Serb *et al.*, 2003
Quadrula pustulosa	AY158767	Mississippi R.	Illinois	Serb *et al.*, 2003
Quadrula pustulosa	DQ640237	FMCC, Virginia Tech	Virginia	Henley *et al.*, 2006
Quadrula pustulosa	DQ640238	FMCC, Virginia Tech	Virginia	Henley *et al.*, 2006
Quadrula pustulosa	DQ640239	FMCC, Virginia Tech	Virginia	Henley *et al.*, 2006
Quadrula pustulosa	DQ640240	FMCC, Virginia Tech	Virginia	Henley *et al.*, 2006
Quadrula pustulosa	DQ640241	FMCC, Virginia Tech	Virginia	Henley *et al.*, 2006
Quadrula quadrula (Rafinesque, 1820)	AY158772	Muskingum R.	Ohio	Serb *et al.*, 2003
Quadrula quadrula	AY158773	Spring R.	Kansas	Serb *et al.*, 2003
Quadrula quadrula	AY158774	Ohio R.	Indiana	Serb *et al.*, 2003
Quadrula quadrula	AY158789	Ohio R.	Kentucky	Serb *et al.*, 2003
Quadrula quadrula	AY158790	Red R.	Kentucky	Serb *et al.*, 2003
Quadrula refulgens (I. Lea, 1868)	AY158788	Pascagoula R.	Mississippi	Serb *et al.*, 2003
Quadrula rumphiana (I. Lea, 1852)	AY158770	Black Warrior R.	Alabama	Serb *et al.*, 2003
Quadrula *rumphiana*	AY158775	Sipsey R.	Alabama	Serb *et al.*, 2003
Quadrula rumphiana	AY158776	Oostanaula R.	Georgia	Serb *et al.*, 2003
Quadrula rumphiana	AY158777	Coosawattee R.	Georgia	Serb *et al.*, 2003
Quadrula sparsa (I. Lea, 1841)	AY158761	Powell R.	Virginia	Serb *et al.*, 2003
Quadrula sparsa	AY158784	Powell R.	Tennessee	Serb *et al.*, 2003
Quincuncina infucata (Conrad, 1834)	AY158795	New R.	Florida	Serb *et al.*, 2003
Quincuncina infucata	AY158810	Ochlocknee R.	Florida	Serb *et al.*, 2003
Truncilla truncata Rafinesque, 1820	AY655125	Mississippi R.	Missouri	Campbell *et al.*, 2005
Tritogonia verrucosa (Rafinesque, 1820)	AY158791	Elk R.	Alabama	Serb *et al.*, 2003
Tritogonia verrucosa	AY158807	Cumberland R.	Tennessee	Serb *et al.*, 2003
Villosa iris (I. Lea, 1829)	AY655127	Duck R.	Tennessee	Campbell *et al.*, 2005

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Comparative morphometry and morphology of glochidial shells of Amazonian Hyriidae (Mollusca: Bivalvia: Unionida)

Daniel Mansur Pimpão[1], Maria Cristina Dreher Mansur[2], Paulo Eduardo Aydos Bergonci[2], and Colin Robert Beasley[3]

[1] Instituto Nacional de Pesquisas da Amazônia - INPA. Coordenação de Pesquisas em Biologia Aquática, caixa postal 478, Manaus, Amazonas, Brazil 69011-970.

[2] UFRGS, Centro de Ecologia, Campos do Vale, Av. Bento Gonçalves 9500, prédio CENECO, CEP 91501-970, Porto Alegre, Rio Grande do Sul, Brazil.

[3] Laboratório de Moluscos, Universidade Federal do Pará, Campus de Bragança, Alameda Leandro Ribeiro, s/n, Aldeia, Bragança, Pará, Brazil.

Correspondence, Daniel M. Pimpão: danielpimpao@yahoo.com.br

Abstract: The glochidia of *Diplodon* (*Diplodon*) *suavidicus* (Lea, 1856), *D.* (*D.*) *obsolescens* F. Baker, 1914, *Diplodon* (*Rhipidodonta*) *hylaeus* (d'Orbigny, 1835), *Prisodon obliquus* Schumacher, 1817, *Paxyodon syrmatophorus* (Meuschen, 1781), *Triplodon corrugatus* (Lamarck, 1819, and *Castalia ambigua* Lamarck, 1819 were redescribed based on comparisons of external morphology and morphometric measurements of larval shells. The outline of the glochidial valves was classified into three types. The morphological comparison was based on light and scanning electron microscopy. Details of the shell, hooks and external sculpture allowed a redescription of the larvae, transferring *D. suavidicus* and *D. obsolescens* to the subgenus *Diplodon* and, within the Prisodontini, the genus *Triplodon* Spix, 1827 was separated from *Prisodon* Schumacher, 1817 and *Paxyodon* Schumacher, 1817. The glochidium of *Prisodon obliquus* is described for the first time. The larvae of the latter species and those of *Paxyodon syrmatophorus* present projections on the external sculpture in the form of spikes that have not yet been seen in the larvae of other South American Hyriidae. This unique ornamentation indicates closer proximity of *Prisodon obliquus* to *Paxyodon syrmatophorus*, a result also supported by multivariate analysis that allowed separation of the glochidia of the different species, using morphometric measurements such as length, height, length of the dorsal line and angle of obliquity formed between the position of the base edge in relation to the center of the dorsal line. Considering the angle of obliquity, the glochidium of *Castalia* Lamarck, 1819 was observed to diverge greatly in relation to those of other genera. The sampling dates of adults containing glochidia in the marsupium and an illustrated glossary are provided.

Key words: glochidium, Hyriinae, larvae, size, South America

Resumo. Morfologia e morfometria comparada das conchas dos gloquídios de Hyriidae da Amazônia (Mollusca, Bivalvia, Unionoida).

Os gloquídios das espécies de Hyriidae da bacia amazônica, *Diplodon* (*Diplodon*) *suavidicus* (Lea, 1856), *D.* (*D.*) *obsolescens* F. Baker, 1914, *Diplodon* (*Rhipidodonta*) *hylaeus* (d'Orbigny, 1835), *Prisodon obliquus* Schumacher, 1817, *Paxyodon syrmatophorus* (Meuschen, 1781), *Triplodon corrugatus* (Lamarck, 1819) e *Castalia ambigua* Lamarck, 1819 são comparados morfologicamente e suas medidas discriminadas por meio de análises multivariadas. Os contornos das valvas gloquidianas foram enquadrados em três padrões esquemáticos. A análise morfológica foi realizada com base em microscopia óptica e de varredura. A visualização dos detalhes da concha e dos ganchos permitiu redescrever as larvas, transferir *D. suavidicus* e *D. obsolescens* para o subgênero *Diplodon* e, dentro de Prisodontini, separar *Triplodon* Spix, 1827 de *Prisodon* Schumacher, 1817 e *Paxyodon* Schumacher, 1817. A larva de *Prisodon obliquus* é descrita pela primeira vez. Os gloquídios desta espécie e de *Paxyodon syrmatophorus* apresentaram projeções esculturais externas sob a forma de espinhos não vistas ainda em outras larvas de Hyriidae sul-americanos. Esta ornamentação exclusiva indicou uma maior proximidade de *Prisodon obliquus* e *Paxyodon syrmatophorus*, resultado corroborado pelas medidas dos gloquídios (comprimento, altura, comprimento da linha dorsal e o ângulo de obliqüidade da extremidade ventral em relação ao centro da base dorsal) conforme a análise multivariada. Este método permitiu também observar maior divergência do gloquídio de *Castalia* Lamarck, 1819 em relação às outras espécies analisadas, considerando a variável ângulo. São fornecidos os períodos de coleta dos indivíduos adultos contendo gloquídios nos marsúpios e um glossário ilustrado.

Palavras-Chave: gloquídio, Hyriinae, larvas, tamanho, América do Sul.

The Naiades, freshwater bivalves of the order Unionoida, or Unioniformes (Starobogatov 1991, Bogan and Roe 2008) or Unionida as recently used by Nevesskaja (2009) and Bieler *et al.* (2010), have a larval stage that is usually a parasite of fish (Wächtler *et al.* 2001). Two types of larvae occur, serving as a basis for the division of the Unionoida into two superfamilies (Parodiz and Bonetto 1963): Unionacea [sic] (= Unionoidea) with glochidia and Mutelacea [sic] (= Muteloidea, part of the current Etherioidea) with lasidia. The Unionoida of South America and Australia that belong to the family Hyriidae Swainson, 1840, according to Ortmann (1921), have glochidia that do not possess microstylets on the base and on the surface of the hooks, which are common in the Unionidae, according to Clarke (1981, 1985).

Glochidial shells of South American Hyriidae are variable in length and are between 200 and 350 μm, depending on the species (Ortmann 1921, Bonetto and Ezcurra 1965, Mansur and Campos-Velho 2000, Vale *et al.* 2005). The glochidial shell has two dorsally articulated valves forming a straight dorsal line. The outline of the valves is subtriangular with a slightly protruding base edge (Ortmann 1921, Bonetto 1961b, Mansur 1999, Mansur and Silva 1999). The larval shell is perforated by numerous micropores that are visible both on the internal and external surfaces (Mansur and Campos-Velho 2000). With high magnification, ridges and depressions, which appear to be organized in a regular manner (Mansur and Campos-Velho 2000), may be observed on the external shell surface. Some species of Hyriidae have a hook on the base edge of each glochidial valve whereas others are hookless (Ortmann 1921, Bonetto 1961b, Parodiz and Bonetto 1963, Mansur and Silva 1999). The hook is present in species with parasitic glochidia (Bonetto 1955) and species of *Diplodon* (*Rhipidodonta*) Mörch, 1853 are hookless (Bonetto 1967). The shape of the hook may be a simple triangle, as in *Castalia* Lamarck, 1819 (Vale *et al.* 2005), or elongated in the form of an "S" with sharp terminal cusps, as in *Triplodon* Spix, 1827 and *Diplodon* (*Diplodon*) Spix, 1827 (Bonetto and Ezcurra 1963, Mansur and Silva 1999). Internally, the glochidia have a very simple arrangement with a single central adductor muscle, phagocytic cells, sensitive cirri, a posterior ciliary organ, and sometimes, a flagellum (Mansur and Campos-Velho 1990, Wächtler *et al.* 2001).

Studying the Amazonian species of Hyriidae, Ortmann (1921) was the first to use glochidia to recognize species, describing the glochidia of *Diplodon hasemani* Ortmann, 1921 [currently *D. hylaeus* (d'Orbigny, 1835), according to Bonetto 1967] and *Castalia acuticosta* Hupé, 1857 [currently *C. ambigua* Lamarck, 1819, according to Bonetto 1967]. Bonetto (1961b) described and figured the outlines of glochidia of 33 species of *Diplodon*, three of which were from the Amazon basin: *Diplodon hartwrighti* (Ihering, 1910), *D. suavidicus* (Lea, 1856) and *D. hasemani*. Later, Bonetto and Ezcurra (1963) described for the first time the glochidium of *Triplodon corrugatus* (Lamarck, 1819), including this species in the genus *Prisodon* Schumacher, 1817. Bonetto (1965), in a revision of the glochidia of the tribe Castaliini, defined the short triangular hook with its sharp distal extremity and, without lateral projections, as an important diagnostic character of the tribe. More recently, Vale *et al.* (2004, 2005) described and quantified variation in the morphology of the valves of glochidia of *Castalia ambigua ambigua* from the Irituia River, Pará. Using scanning electron microscopy, the authors observed for the first time, the presence of a protuberance on the ventral base of the glochidial hook. Beasley *et al.* (2005) determined the brood size and larval length during the reproductive season (February to September) of *Paxyodon syrmatophorus* (Meuschen, 1781) from the Tocantins River. From the Orinoco basin in Venezuela, the glochidia of *Castalia ambigua multisulcata* Hupé, 1857 (synonym of *Castalia orinocensis* Morrison, 1943, according to Mansur (1991) were described by Martínez (1983) and *Diplodon* (*D.*) *granosus granosus* (Bruguière, 1792) described by Martínez and Royero (1995). Specimens of the latter species have been collected from the Amazon basin (*pers. obs.*).

According to Bonetto (1961b), the morphological characteristics of glochidia are not relevant for distinguishing between species, regardless of being parasitic or not. However, the morphology of the glochidium may have potential as a taxonomic tool, at both species (Mansur and Campos-Velho 1990, Jupiter and Byrne 1997, Mansur 1999) and higher taxon levels (Bonetto 1960, 1961b, Bonetto and Ezcurra 1965, Graf 2000, Sayenko *et al.* 2005). Parada *et al.* (1989) emphasized the importance of morphology and morphometrics of the glochidial shell, as well as the shape and structure of the hooks as distinguishing characters. Greater attention is being given to glochidial morphology, especially fine structure in taxonomy (Wu *et al.* 1999). The present study aims to describe the larvae of the poorly-known hyriid fauna from the Amazon basin by means of a morphological and a morphometric comparison of the glochidia of seven species and five genera as a contribution to the taxonomy of the family.

MATERIAL AND METHODS

Gravid females (with larvae on the external demibranch of the marsupia) were collected by hand from shallow banks and margins of rivers of the Brazilian part of the Amazon. Sampling was carried out during or at the end of the dry season, when the animals are closer to the water's edge and manual sampling is feasible. After capture, the bivalves were maintained for 24 h in river water to which were added crystals of pure menthol ($C_{10}H_{20}O$) in order to relax the animals. Afterwards, the specimens were preserved in 70% ethanol. In some cases, the larvae were removed before fixation and maintained in distilled water for up to five days, in order to macerate the soft parts in preparation for scanning electron microscopy (SEM).

The following Hyriidae that occur in the Amazon basin were included in the analysis: *Diplodon*, *Triplodon*, *Paxyodon* Schumacher, 1817, *Prisodon*, and *Castalia*. Both subgenera of *Diplodon*, *Diplodon* (*Diplodon*) with the glochidial hook in the form of an "S" and with a protuberance at the base of the hook, and *Diplodon* (*Rhipidodonta*), with a hookless glochidium without a protuberance, were also included. Some specimens of *D. suavidicus*, from the Jamari River (Rondonia State), were deposited in the Mollusc Collection at the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus. Specimens of other species were deposited at INPA and in the Laboratório de Moluscos, Universidade Federal do Pará, Campus de Bragança. Larvae from adults that had been fixed in 70% ethanol were removed from the marsupia and hydrated in a series of solutions of ethanol of increasing water content, ending in distilled water. Soft parts were removed with a solution of sodium hypochlorite (four drops of NaClO in 10 ml of water). The cleaning process was monitored under the stereoscope. Once the tissues had been removed, the valves were rinsed four times in distilled water to eliminate the sodium hypochlorite. Afterwards, the material was dehydrated, as described by Mansur and Campos-Velho (1990), in order to mount permanent light microscope slides as well as stubs for SEM and photography.

To avoid crushing the glochidia with the weight of the coverslip during the mounting process of the permanent slides, each group of larvae was surrounded by a single layer of fine strips of paper before sealing the coverslip to the slide with Entellan®. Glochidia were photographed using an Olympus® light microscope digital photography system. The free software CombineZM (2009) was used to obtain focal integration for some figures. A Philips® XL30 and a JEOL® JSM6060 electron microscopes were used to carry out scanning electron photomicroscopy.

To ease the redescription and comparison of glochidia of the species studied, the outlines of the larvae were classified according to three schemes based on the type of triangle: equilateral, isosceles and scalene (Fig. 1). The definition of the anterior and posterior regions of the glochidial valves follows that of Hoggarth (1987). The term spike was based on Callil and Mansur (2005).

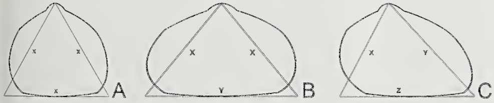


Figure 1. Schematic drawings of the three basic shapes of Amazonian glochidia, based on triangles. A, Equilateral, with all three sides approximately equal. B, Isosceles, with two sides of equal length and a generally longer dorsal line. C, Scalene, with all three sides of unequal lengths and the base edge displaced from the center. Letters represent hypothetical measurements and sides with the same letter are of the same length.

Larval shells that were in a maximal horizontal position were drawn and measured under the light microscope for length, height, and dorsal line length (in µm) and the angle of obliquity (measured in °). The angle of obliquity is defined as the angle of the ventral point in relation to the center of the dorsal line (Mansur and Campos-Velho 1990), i.e. the angle between the line that joins the ventral point to the middle of the hinge and the line that joins the ventral point perpendicular to the hinge.

The terminology referring to the glochidial hook was standardized and defined in a glossary (Table 1). The data and illustrations of Vale *et al.* (2005) were added to the description of the glochidium of *Castalia ambigua*.

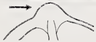
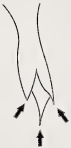
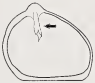
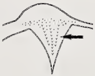
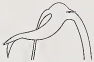
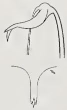
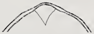
Variability in glochidium length, height, dorsal line length and the angle of obliquity were analyzed using canonical discriminant analysis (CDA). Groups of larvae are distinguished by CDA which aims to remove the influence of size to allow discrimination among shapes. The multivariate analysis was carried out on natural log transformed data using SPSS 13.0.

Material examined: *Diplodon* (*Diplodon*) *suavidicus*. BRAZIL, **Amazonas:** Novo Aripuanã (Aripuanã River, downstream of Pernambuco Beach 06°01′05.1″S 60°11′39.5″W), 1 specimen, 06.IX.2007, D. M. Pimpão *leg.* (INPA 1273); (Pernambuco beach 06°01′05.1″S 60°11′39.5″W), 1 specimen, 05.IX.2007, D. M Pimpão *et al. leg.* (INPA 1260); 10 specimens, 06.IX.2007, D. M Pimpão *et al. leg.* (INPA 1265); (Aripuanã River 06°01′29.3″S 60°11′35.8″W), 1 specimen, 13.IX.2004, D. M Pimpão and C. Sotero *leg.* (INPA 326); **Pará:** (Xingú River, Neusoranga Beach, 03°27′56″S 51°57′31″W, 1 specimen, 25. XI.2000, C. R. Beasley col. (INPA 1585); **Rondônia:** (Jamari River, Usina Hidrelétrica Samuel, 08°44′S 63°27′W), 1 specimen, 21.VIII.1987, C. S. Motta *et al. leg.* (INPA 205).

Diplodon (*Diplodon*) *obsolescens*. BRAZIL, **Mato Grosso:** Aripuanã, Aripuanã River, upstream of the Dardanelos waterfall, 10°09′49.7″S 59°27′45.9″W, 31 specimens, 06.VII.2007, D. M Pimpão *et al. leg.* (INPA 1235).

Diplodon (*Rhipidodonta*) *hylaeus*. BRAZIL, **Mato Grosso:** Aripuanã (Aripuanã River, upstream of the Dardanelos waterfall, 10°10′08.6″S 59°27′55.9″W) 6 specimens, 05.VII.2007, D. M Pimpão *et al. leg.* (INPA 1230); 2 specimens, 09.VII.2007, D. M Pimpão *et al. leg.* (INPA 1249); 6 specimens,

Table 1. Terms used to describe characteristics of glochidia of the Unionoida. ES, Spanish; PT, Portuguese.

Term adopted	Definition	Terms found in the literature / author	Schematic drawing	Taxa having the characteristic
Base edge	Ventral projection of the glochidia	Ventral edge: Clarke 1981		*Diplodon* (*Diplodon*), *Prisodon*, *Triplodon*, *Castalia*
Cusps	Sharp terminations of the glochidial hooks. A central, more elongated one directed towards the interior and two short lateral ones on the sinuous hook; a single sharp cusp or in the shape of a beak in the triangular hook.	Cúspides (PT): Mansur 1999, Mansur and Campos-Velho 1990 Cusps: Mansur and Silva 1999 Espinas (ES): Bonetto *et al.* 1986, Martínez 1983, Martínez and Royero 1995 Espinhos (PT): Mansur and Campos-Velho 1990 Espínulas (ES): Bonetto 1960b, Bonetto and Ezcurra 1965, Bonetto *et al.* 1986, Martínez and Royero 1995 Lancetas (ES): Bonetto 1960b, Martínez 1983 Lancets or microstylets: Mansur and Campos-Velho 2000 Punta aguda (ES): Martínez 1983		Unionidae, *Diplodon* (*Diplodon*), *Prisodon*, *Triplodon*, *Castalia*
Hook	Distal projection close to the ventral extremity and directed towards the inside of the glochidial shell. Triangular shape or elongate with wide base.	Dente (PT): Mansur 1999, Mansur and Campos-Velho 1990 Diente (ES): Bonetto 1954, 1955, 1961, Bonetto 1960b, Bonetto *et al.* 1986, Bonetto and Ezcurra 1965, Martínez 1983, Martínez and Royero 1995 Hook: Ortmann 1921, Kinzelbach and Nagel 1986, Jupiter and Byrne 1997, Wächtler *et al.* 2001, Vale *et al.* 2005 Spine: Mansur and Campos-Velho 2000 Stylet: Clarke 1985 Tooth: Mansur and Silva 1999		Unionidae, *Diplodon* (*Diplodon*), *Prisodon*, *Triplodon*, *Castalia*
Microstylets	Small projections in the form of spines found on the base and on the surface of the hooks.	Microstylet: Clarke 1985 Spines: Kinzelbach and Nagel 1986, Jupiter and Byrne 1997 Spinules: Ortmann 1921 Tooth: Wächtler *et al.* 2001		Unionidae
Protuberance	Rounded projection originating from the base of the hook.	Prominence: Mansur and Silva 1999 Protuberance: Vale *et al.* 2005 Tooth: Jupiter and Byrne 1997		*Diplodon* (*Diplodon*), *Castalia*, Prisodontini
Sinuous hook	Elongate projection, in the shape of an "S" viewed from the front of the valve, with three terminal cusps. In lateral view, the hook has the shape of an anchor, with a wide base* (arrow).	Dente em forma de S (PT): Mansur 1999, Mansur and Campos-Velho 1990 Diente en forma de S (ES): Bonetto *et al.* 1986 Diente en forma sigmoidea (ES): Bonetto and Ezcurra 1965 S-shaped tooth: Mansur and Silva 1999, Mansur and Campos-Velho 1990		*Diplodon* (*Diplodon*), *Prisodon*, *Triplodon*
Triangular hook	Triangular projection with wide base and a single cusp on the distal extremity. Cusp sharp or in the shape of a beak.	Dente triangular: Mansur and Campos-Velho 1990 Diente agudo (ES): Martínez 1983 Diente triangular (ES): Bonetto *et al.* 1986 Triangular hook: Vale *et al.* 2005 Triangular spine: Mansur and Campos-Velho 2000		Unionidae, *Castalia*

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* Wu *et al.* (1999) denominate as "wings" what is here called the base.

05.VII.2007, D. M Pimpão *leg.* (INPA 1234); PERU, **Ucayali:** Pucallpa (Quebrada Durand, 08°23′S 74°33′W), 1 specimen, 13.IX.1996, K. Valdivia *leg.* (INPA 1363).

Triplodon corrugatus. BRAZIL, **Roraima:** (Branco River, 01°02′S 61°51′W) 9 specimens, 04.VII.2003, N. L. Chao *et al. leg.* (INPA 1074); **Pará:** Santarém (Alter do Chão, Tapajós River, 02°30′27.2″S 54°57′54.8″W) 1 specimen, 12.XI.2006, D. M Pimpão *et al. leg.* (INPA 1183).

Paxyodon syrmatophorus. BRAZIL, **Pará:** Santarém (Alter do Chão, Tapajós River, 02°30′41.2″S 54°57′59.3″W) 6 specimens, 03.X.2007, D. M Pimpão *leg.* (INPA 1355); (02°30′S 55°00′W), 1 specimen, 16.XI.1992, C. C. Fernandes *leg.* (INPA 1432).

Prisodon obliquus. BRAZIL, **Amazonas:** Novo Aripuanã (Aripuanã River, Pernambuco Beach, 06°01′05.1″S 60°11′39.5″W) 19 specimens, 06.IX.2007, D. M Pimpão *et al. leg.* (INPA 1264); 8 specimens, 07.IX.2007, D. M Pimpão and M. S. Rocha *leg.* (INPA 1288).

Castalia ambigua. BRAZIL, **Pará:** Irituia (Irituia River, in front of Irituia town 01°46′11.1″S 47°26′5.9″W - Station B, and 2 km downstream of Irituia town - Station A), 19–21.V.2001, R. Souza do Vale *leg.* (UFPA Bragança, no number).

RESULTS

Description of the glochidia

BIVALVIA
HETEROCONCHIA
HYRIIDAE
Glochidium type larvae with or without hooks

Diplodontini Parodiz and Bonetto, 1963
Diplodon (*Diplodon*) Spix, 1827
Glochidium with hook in the form of an "S" and protuberance present.

Diplodon (*Diplodon*) *suavidicus* (Lea, 1856)

Outline similar to a scalene triangle (Figs. 2A, 4A), length (ave. = 291.4 µm) greater than height (ave. = 246.5 µm); base edge pointed and conspicuous, displaced from the center (mean angle of obliquity = 14.7°); valves inequilateral, anterior margin greater and more arched than posterior; external and internal surfaces presenting subtle relief formed by microdepressions and elevations, similar to the surface of an egg-shell, without any apparent orientation; radial grooves and pores may be seen (diameter of 1 to 2 µm) in the center of the depressions of the external surface that cross the valves and are visible on the apex of elevations on the internal surface; valve border without pores, nearly smooth, presenting microscopic delicate perpendicular folds close to the margin; seen from the side, hook in the form of an anchor and with a

Table 2. Comparison of morphometric variables of the glochidia of seven species of Hyriidae from the Amazon. Â, angle of obliquity; Ave., average; CV, coefficient of variation, **L**, length, **H**, height, **DLL**, dorsal line length, **SD**, standard deviation. All variables expressed in µm except for Â, which is expressed in degrees (°).

	L				H				DLL				Â			
Species	Max/min	Ave.	*SD*	CV (%)	Max/min	Ave.	*SD*	CV (%)	Max/min	Ave.	*SD*	CV (%)	Max/min	Ave.	*SD*	CV (%)
Triplodon corrugatus	298.7 251.9	273.3	11.4	0.04	260.8 217.0	237.7	9.09	0.04	210.7 154.9	179.2	11.32	0.06	16 7.5	12.0	1.73	0.14
Paxyodon syrmatophorus	254.5 223.0	242.5	5.33	0.02	198.9 167.6	187.0	5.13	0.03	194.6 151.3	178.9	6.79	0.04	7.0 0	3.5	1.33	0.38
Prisodon obliquus	251.4 232.1	241.5	5.18	0.02	182.2 164.8	174.1	4.37	0.02	202.0 168.6	186.7	7.62	0.04	7.0 1.0	4.7	1.64	0.35
Castalia ambigua	282.0 251.9	262.7	6.0	0.02	271.9 246.6	258.5	5.15	0.02	192.9 166.8	181.3	5.93	0.03	6.0 0	2.2	1.58	0.73
Diplodon hylaeus	312.9 275.4	293.6	7.07	0.02	252.2 222.7	238.8	5.87	0.02	225.2 169.0	194.8	10.52	0.05	21.0 12.0	16.3	1.64	0.10
Diplodon suavidicus	302.4 279.6	291.4	5.16	0.02	266.2 229.3	246.5	6.75	0.03	220.6 181.6	204.2	8.01	0.04	18.5 11.5	14.7	1.37	0.09
Diplodon obsolescens	334.9 303.3	318.8	9.17	0.03	280.6 252.4	265.4	10.05	0.04	234.0 193.3	216.7	13.21	0.06	12.0 6.0	8.1	1.77	0.22

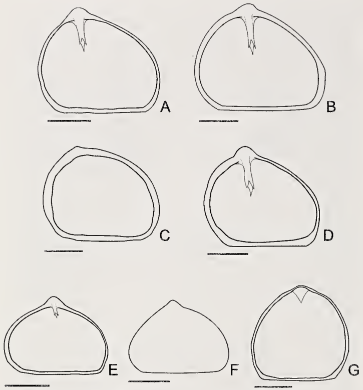


Figure 2. Outlines of glochidia of Amazonian Hyriidae. **A,** *Diplodon suavidicus.* **B,** *Diplodon obsolescens.* **C,** *Diplodon hylaeus,* a hookless glochidium. **D,** *Triplodon corrugatus.* **E,** *Paxyodon syrmatophorus.* **F,** *Prisodon obliquus,* incompletely formed glochidium, as yet without a complete hook. **G,** *Castalia ambigua* Scale bar = 100 μm.

wide triangular base, which narrows gradually and straightens in the center; three terminal cusps occur at the distal extremity, two are smaller, one on each side, and a longer sharp central cusp, pointing towards the inside of the valve, with the distal extremity directed towards the dorsal line; seen from the front, the hook has an "S" shape (Figs. 3A, 4B–4C), the hook is open, in the form of a groove, on the internal face, which allows the opposite hook to fit when the valves close; hook length (ave. = 90.6 μm) approximately one and a half to two times the width of the base; triangular protuberance with rounded apex, projected between the ventral base of the hook and the base edge as though a prolongation of itself, directed towards the inside of the valve, sometimes absent with a foramen in its place. Usually a more robust protuberance seen on one valve and a foramen on the other, sometimes with a small protuberance. Gravid females were collected in August (falling to low water period in the Jamari River, Roraima state) and September (falling water period ["*descida das águas*"] in the middle to lower reaches of the Aripuanã River, Amazonas state).

Diplodon (*Diplodon*) *obsolescens* F. Baker, 1914

Outline similar to a scalene triangle (Figs. 2B, 4D), length (ave. = 318.8 μm) greater than height (ave. = 265.4 μm); base edge pointed and displaced from the center (mean angle of obliquity = 8.1°), valves inequilateral, anterior margin taller and more arched than posterior. External and internal surfaces presenting subtle relief formed by microdepressions and elevations, similar to the surface of an eggshell, with apparent commarginal orientation; presence of radial grooves and pores (diameter of 1 to 2 μm) in the center of the depressions of the external surface that cross the valves and are visible on the apex of elevations on the internal surface; larger pores are concentrated in the central part of the valves along with the adductor muscle scar; valve border without pores and apparently smooth, with microscopic delicate perpendicular folds close to the margin. Seen from the side, hook in the form of an anchor, with a wide triangular base, the margins of which are concave on immediately projecting from the base (border in this case), after which they proceed straight towards the distal end of the hook, which has three terminal cusps, two are smaller, one on each side, and a longer central sharp cusp directed towards the inside of the valve, with the distal extremity directed towards the dorsal line. Seen from the front of the glochidium, the hook has an "S" shape (Figs. 3B–3C, 4E); on the internal face the hook is open, in the form of a groove, which allows the opposite hook to fit, when the valves close. Hook length (ave. = 114.1 μm) approximately twice the width of the base; protuberance projected from the ventral base of the hook, as a continuation of the base edge, with the distal edge rounded or flattened, sometimes a foramen is present. Gravid females were collected in July (falling water period on the upper Aripuanã River, Mato Grosso state).

Diplodon (*Rhipidodonta*) Mörch, 1853
Glochidium without either hook or protuberance.

Diplodon (*Rhipidodonta*) *hylaeus* (d'Orbigny, 1835)

Outline similar to a scalene triangle (Figs. 2C, 4F), length (ave. = 293.6 μm) greater than height (ave. = 238.8 μm), base edge rounded, without a projection, displaced from the center (mean angle of obliquity = 16.3°). Valves strongly inequilateral, anterior margin almost as convex as posterior. Pores that pass through valves visible on the internal (Fig. 3D) and external surfaces. Glochidium without either a hook or a protuberance. External surface with a very evident relief formed by deep elevations and depressions with apparent commarginal orientation; these elevations present a rounded apex and the sides are grooved, the grooves (numbering 3 to 5) converge to the pores in the center of the depressions, the structure is reminiscent of upholstered material, but without a regular pattern. Some of these grooves also advance over the borders of the valve, which do not present pores, forming regularly spaced groups of divergent radial microfurrows/grooves; internally

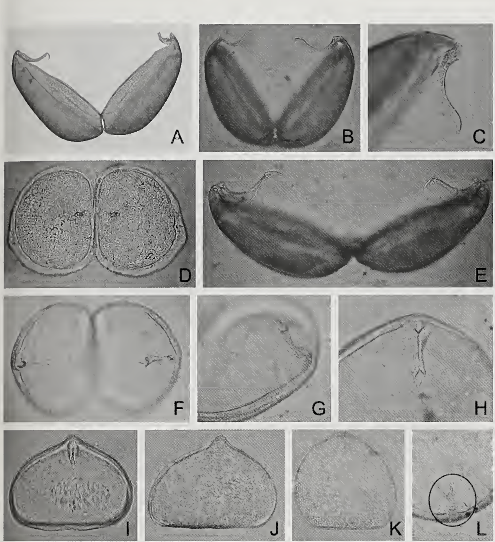


Figure 3. Photomicroscopy of the glochidia of Amazonian Hyriidae. **A**, *Diplodon suavidicus*, frontal view of valves with profile of hook. **B–C**, *Diplodon obsolescens*. **B**, Frontal view of valves. **C**, Profile of hook. **D**, *Diplodon hylaeus*, lateral view, valves open. **E–H**, *Triplodon corrugatus*. **E**, Frontal view of the valve. **F**, Ventral view. **G**, Hook in latero-ventral view. **H**, Ventral view of hook. **I**, *Paxyodon syrmatophorus*, lateral view of valve. **J**, *Prisodon obliquus*, lateral view of valve. **K–L**, *Castalia ambigua*. **K**, Lateral view of valve. **L**, Ventral view of hook. Magnification 400 X.

the border of the valves is smooth, wide and very much detached from the rest of the internal surface (Fig. 4G) that presents rounded elevations topped by pores. Gravid females were collected in July (falling water period on the upper Aripuanã River, Mato Grosso state).

Prisodontini Modell, 1942

Triplodon Spix, 1827

Glochidium with a hook in the shape of an "S" and presence of a protuberance; characteristics similar to those of species of *Diplodon* (*Diplodon*).

***Triplodon corrugatus* (Lamarck, 1819)**

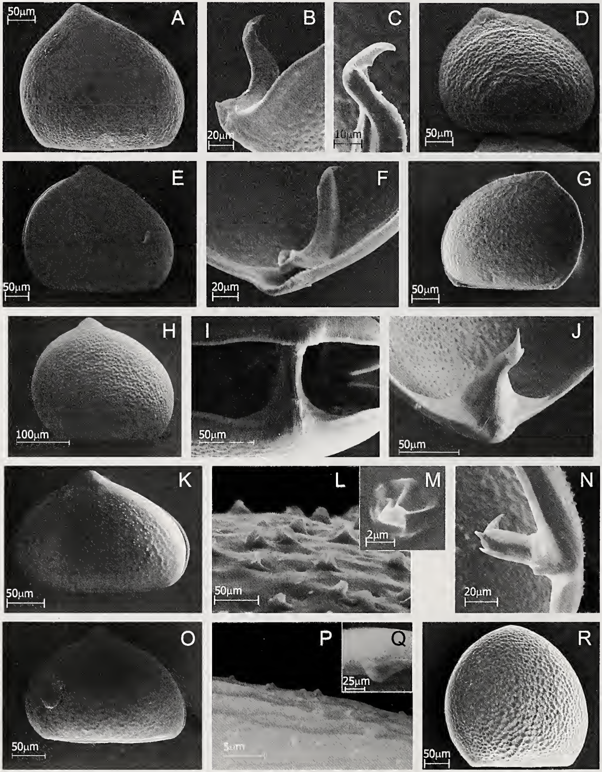
Outline similar to a scalene triangle (Figs. 2D, 4H), length (ave. = 273.3 µm) greater than height (ave. = 237.7 µm), base edge pointed and displaced from the center (mean angle of obliquity = 12.0°); valves inequilateral, anterior margin taller and more arched than posterior; pores that cross the valves visible on the internal and external surfaces. External surface with a very delicate relief consisting of chains of rounded depressions with a central pore, forming a fine and densely dotted pattern that is reminiscent of an eggshell surface, with an apparent commarginal orientation; valve border filled with more or less parallel microfolds, perpendicular to the border, also present on the protuberance and on the hook (Fig. 4I); reinforced hook, with a very wide base; in the form of an anchor in lateral view (Figs. 3F, 3H), narrows toward distal end and proceeds straight along the central part, as it approaches the three terminal cusps: one central, elongate and projected towards the dorsal line of the valve, and two small, short laterals (Fig. 4J); seen from the front the hook has an "S" shape (Figs. 3E, 3G); the internal face is open as in species of *Diplodon* (*Diplodon*) described above; hook length (ave. = 102.5 µm) approximately one and a half times the width of the base; protuberance projected from the ventral base of the hook, with rounded extremity. Gravid females collected in November (falling to low water period in the lower Tapajós River, Pará state), July (high water on the lower Branco River, Roraima state) and May (end of high water period on the Pará River, Melgaço, Pará state).

Paxyodon Schumacher, 1817

Glochidium with short and straight to slightly sinuous hook, protuberance present; external surface with microprojections in the form of spines.

***Paxyodon syrmatophorus* (Meuschen, 1781)**

Outline similar to an isosceles triangle (Figs. 2E, 4K), length (ave. = 242.5 µm) greater than height (ave. = 187.0 µm); base edge pointed and slightly displaced from the center (mean angle of obliquity = 3.5°); valves nearly equilateral (Fig. 3I), anterior margin slightly taller and more arched than posterior. External surface presenting shallow depressions and subtle elevations, reminiscent of a slightly hammered


A
50µm
B
20µm
C
10µm
D
50µm
E
50µm
F
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G
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H
100µm
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J
50µm
K
50µm
L
50µm
M
2µm
N
20µm
O
50µm
P
5µm
Q
25µm
R
50µm

Figure 4. Photomicrography of the glochidia of Amazonian Hyriidae. **A–C**, *Diplodon suavidicus*. **A**, Lateral view of the valve. **B–C**, Hook in detail. **D–E**, *Diplodon obsolescens*. **D**, Lateral view of the valve. **E**, Internal-ventral view of hook. **F–G**, *Diplodon hylaeus*, valves. **F**, External lateral view. **G**, Internal view. **H–J**, *Triplodon corrugatus*. **H**, Lateral view of the valve. **I**, United hooks and protuberance at ventral basis of hook. **J**, Ventral view of the hook. **K–N**, *Paxyodon syrmatophorus*. **K**, Lateral view of the valve. **L**, Detail of the external surface with spikes. **M**, Detail of a spike. **N**, Ventral view of the hook. **O–Q**, *Prisodon obliquus*. **O**, Lateral view of the valve. **P**, External surface in detail with spikes. **Q**, Detail of hook formation. **R**, *Castalia ambigua*, lateral view of the valve.

surface; depressions with one or two pores that cross the valves; presence of microprojections as spikes (Fig. 4L) with a rounded distal extremity and a palmate base, whose extremities form structures similar to anchors (Fig. 4M); basal projections of spikes generally number three to five; valve border with perpendicular microfolds, less numerous and wider on the protuberance; microfolds also present on the base of the hook; at high magnifications microgranules may be observed on the borders and on the hook. Hook in the form of an anchor in lateral view, with a wide triangular base that tapers gradually towards the central region, relatively short, three cusps on the distal extremity, two smaller ones, one on each side, and a longer sharp central cusp, projected towards the dorsal line (Fig. 4N); hook length (ave. = 62.8 µm) approximately equal to the width of the base; hook almost straight seen from the front, slightly inclined inwards (groove not seen); protuberance projected from the ventral base of the hook (Fig. 4N), with rounded distal extremity. Gravid females collected in October (falling water period on the lower Tapajós River, Pará state) and May (end of high water period on the Pará River, Melgaço, Pará state).

Prisodon Schumacher, 1817

Glochidium with a hook, external surface with microprojections in the form of spikes.

***Prisodon obliquus* Schumacher, 1817**

Outline similar to an isosceles triangle (Figs. 2F, 3J, 4O), length (ave. = 241.5 µm) greater than height (ave. = 174.1 µm); base edge pointed in the center (half the valve length) or slightly displaced from the center (mean angle of obliquity = 4.7°); valves nearly equilateral, anterior and posterior margin equally convex. External surface with a very subtle relief of depressions and elevations forming a hammered pattern; pores (one to two) that cross the valves, visible on the internal and external surfaces; microprojections similar to spikes (Fig. 4P) with a rounded distal extremity and a palmate base; basal projections of spikes generally number from three to five; as in *Paxyodon syrmatophorus*; borders with perpendicular microfolds and punctuations. The glochidia obtained presented an externally complete and well-formed shell, the hooks present were, however, still being formed. The formation of the protuberance and the outline of the hook were observed in an incomplete specimen with slightly deflected borders (Fig. 4Q). Gravid females were collected in September (falling water period in the middle to lower Aripuanã River, Amazonas state).

Castaliini Parodiz and Bonetto, 1963

Castalia Lamarck, 1819

Glochidium with triangular hook slightly curved with beak, protuberance present.

***Castalia ambigua* (Lamarck, 1819)**

Outline similar to an equilateral triangle (Figs. 2G, 3K, 4R), length (ave. = 262.7 µm) similar to height (ave. = 258.5 µm); base edge pointed in the center (mean angle of obliquity = 2.2°). Valves nearly equilateral, anterior and posterior margins equally convex. External surface with a relief formed by rows of depressions and elevations with an apparently commarginal orientation similar to a hammered pattern; depressions with pores (from one to three) that cross the valves. Valve border without pores and apparently smooth, however, at high magnifications, delicate perpendicular folds may be seen that anastomose in an irregular fashion. Triangular hook uniform with a wide base in lateroventral view (Fig. 3L), lateral margins almost straight and convergent on the distal extremity; in frontal view, the hook is curved inwards resembling a beak; hook length (ave. = 43.7 µm) approximately equal to the width of the base; a single cusp on the rounded or flattened distal extremity. Protuberance and hook with occasional microfolds similar to those at the borders. Gravid females were collected in May (end of high water period on the Irituia River, Pará state). (In part Vale *et al.* 2005).

Multivariate analysis

Canonical Discriminant Analysis

Glochidia of each genus were distinguished by CDA. Of the total variability, 82.2% is explained by Function 1 in which height is the principal variable responsible (0.903). Function 2 represents 14.8% of the variation, influenced by the angle of obliquity (0.613).

CDA resulted in the formation of three groups (Fig. 5): the first is composed of *Prisodon obliquus* and *Paxyodon syrmatophorus*; the second includes *Triplodon corrugatus* and the three species of *Diplodon*; and the third representing only *Castalia ambigua*. Although the glochidia of *Prisodon obliquus* and *Paxyodon syrmatophorus* were most similar between themselves than in comparison with those of the other species, both were clearly distinguished in the analysis (see centroid, Fig. 5). The *Prisodon-Paxyodon* group was separated from the others by the height of the glochidial shell (Function 1). *Triplodon corrugatus* was more similar to the three species of *Diplodon*. However, these four species were also clearly distinguished in the analyses (see centroids). Finally, *Castalia ambigua* was the species that was most distinct from all the others in terms of the glochidial shell shape, as a result of the angle of obliquity (Function 2).

DISCUSSION

Both morphological descriptions and morphometric analyses were efficient in identifying and distinguishing among genera and species and in the classification of supra

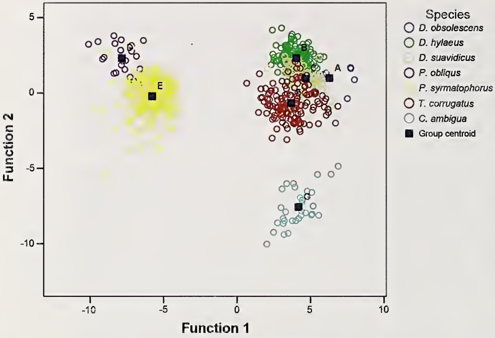

Figure 5. Canonical Discriminant Analysis of the morphometry of glochidial shells of Amazonian hyriid species: *Diplodon suavidicus, D. obsolescens, D. hylaeus, Prisodon obliquus, Paxyodon syrmatophorus, Triplodon corrugatus* and *Castalia ambigua*, showing three distinct groups of larvae.

specific taxa. The analysis separated the larvae into three groups that coincide with the three basic shapes of Amazonian glochidia of Hyriidae.

The presence of spikes, seen using SEM, on the external surface of glochidia of *Prisodon obliquus* and *Paxyodon syrmatophorus*, here described for the first time, but absent in other South American species (Martínez 1983, Martínez and Royero 1995, Mansur and Silva 1999, Mansur and Campos-Velho 2000, Vale *et al.* 2005), including *Triplodon*, constitutes a unique characteristic of *P. obliquus* and *P. syrmatophorus*, in terms of ultrastructural larval morphology. Based on the morphology of the hinge of adult shells, Olsson and Würtz (1951) grouped *P. obliquus* with the other member of the Prisodontini, *T. corrugatus*, both distinct from *Paxyodon syrmatophorus*. This classification was followed by Simone (2006). However, the similarity between *P. obliquus* and *P. syrmatophorus* is supported here by glochidium morphometrics, in which both species form a well defined and consistent group that may suggest their union under the genus *Prisodon*, distinct from *Triplodon*. A cladistic study (Pimpão 2010), using adult shell and soft parts, provides further evidence for affinities between *Prisodon* and *Paxyodon*.

Diplodon and *Triplodon* have, up to now, always been separated on the basis of adult shell characteristics alone. The presence of posterior and anterior alate projections on the shell is diagnostic of the Prisodontini, despite the fact that smaller specimens have reduced projections. Morphometric analysis and ultrastructural observations from the present study provide evidence for the morphological similarity of the larvae of *Diplodon* spp. and *Triplodon corrugatus*.

Glochidia belonging to the genus *Diplodon* were either hooked or hookless, a character that allowed the separation of one of the species, *D. hylaeus*. Ortmann (1921), Bonetto (1961b) and Haas (1969), also using the presence/absence of the glochidial hook, separated *Diplodon* into two subgenera: *Diplodon* (*Diplodon*) and *D.* (*Rhipidodonta*). In a survey of larvae of *Diplodon*, Bonetto (1961b) measured and described the glochidium of *D. suavidicus* as being non-parasitic. In the description the author informs that the larvae were from a single specimen from the upper Xingu River, Mato Grosso state. The hookless glochidia in the present study are from a nearby region (upper Aripuanã River) in the same state (Mato Grosso) and were identified as *Diplodon hylaeus*. Ortmann (1921) also mentions that this species [sic] (*D. hasemani*) has non-parasitic glochidia. Shells of adult *D. hylaeus* from the upper Aripuanã River have very worn umbos and faintly marked shell sculpture. This may possibly have caused Bonetto (1961b) to misidentify *D. hylaeus* from the upper Xingu as *D. suavidicus*, which has a similarly weak shell sculpture. Morphometric analysis show that both species overlap somewhat on the ordination, despite the centroids occupying separate positions.

The first author to question the validity of the species *Diplodon obsolescens* and *D. suavidicus* was Bonetto (1960, 1961b) who considered both as synonyms. Simone (2006) raised the subgenus *Rhipidodonta* to the level of genus and maintained the above mentioned Amazonian species as synonyms in the genus *Rhipidodonta*, despite the revalidation of *D. obsolescens* by Mansur and Valer (1992) on the basis of a comparison of morphological characteristics of the shells. Pimpão and Mansur (2009) also distinguished between *D. obsolescens* and *D. suavidicus* on the basis of adult shell characteristics. In the present study, glochidia of both species were found to be hooked. Since the presence of glochidial hooks is a diagnostic characteristic of the subgenus *Diplodon senso stricto*, it is suggested here that both species should be united in *Diplodon* (*Diplodon*). The smaller larval shell length of *D. suavidicus* (ave. = 291.4 µm) allows it to be distinguished from the larger glochidium of *D. obsolescens* (ave. = 318 µm). Subgenera of *Diplodon* are distinguished only according to the presence of glochidial tooth in *Diplodon* (*Diplodon*) or absence in *D.* (*Ripidodonta*). Simone (2006) erected the subgenus to a higher level (genus) without providing any diagnostic characters. Considering that glochidia of many Diplodotini species are still unknown, and that criteria based on adult shell and soft parts are lacking for *Rhipidodonta*, it was an option of these authors to maintain it at the subgeneric level, until new studies could evidence the differences between both genera.

In terms of shape, the glochidium of *Castalia ambigua* was distinct from those of all the other species due to differences in the angle of obliquity, which, according to Vale *et al.* (2005), the obliquity is close to zero. Other authors have also observed this striking characteristic of species of Castaliini (Bonetto 1961a, 1965, Martínez 1983).

CONCLUSION

The morphometric comparison of the outline, sculptural patterns, and hooks of glochidial shells provide further evidence for the maintenance of the two subgenera in *Diplodon* and, within the Prisodontini, indicate the separation of *Triplodon* from *Prisodon* and *Paxyodon*. The external surface sculpture ornamentation of the glochidia with the presence of spikes, seen only under SEM, and described for the first time, reinforces this separation.

The morphometric comparison, using multivariate analyses, allowed separation of the species of Hyriidae into three groups that coincide with the three basic shapes of Amazonian glochidia.

The larvae of *Castalia* show wide divergence in relation to the other genera, principally with respect to the angle of obliquity in relation to the base edge.

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A revision of the living Mactridae (Bivalvia: Autobranchia) from Northern Argentina and Uruguay

Javier H. Signorelli[1] **and Guido Pastorino**[2]

[1]Centro Nacional Patagónico, CONICET Boulevard Brown 2915, U9120ACD. Puerto Madryn, Chubut, Argentina
[2]Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" Ángel Gallardo 470 C1405 DJR, Buenos Aires Argentina
Correspondence, Javier H. Signorelli: jsignorelli@cenpat.edu.ar

Abstract: Living bivalves of the family Mactridae from Northern Argentina and Uruguay are herein revised. A bibliographical research yielded 22 nominal species reported from this area. Material from several institutions as well as fieldwork from La Paloma, Uruguay to San Blas, Argentina was studied. In addition, all type material were illustrated and redescribed. Descriptions of the soft and hard parts including ultrastructure of the shell, hinge and ligament using the Scanning electronic microscope (SEM) were carried out. We consider *Mactra guidoi* Signorelli and Scarabino, 2010, *Mactra isabelleana* d'Orbigny, 1846, *Mactra marplatensis* Doello-Jurado, 1949, *Mactra petitii* d'Orbigny, 1846, *Mactrella janeiroensis* (Smith, 1915), *Raeta plicatella* (Lamarck, 1818) and *Darina solenoides* (King, 1832) as valid species. In addition, a list of synonyms as well as the corroborated distribution is proposed for each species.

Key words: Southwestern Atlantic, *Mactra, Darina, Raeta, Mactrella*

The family Mactridae includes five subfamilies: Mactrinae Lamarck, 1809; Lutrariinae Gray, 1853; Kymatoxinae Stenzel *et al.*, 1957; Darininae Signorelli in Carter *et al.* 2011 and Tanysiphoninae Scarlato and Starobogatov, 1971. The latter group, described as a family, was recently considered as a subfamily of Mactridae (Bieler *et al.* 2010). Only Mactrinae, Kymatoxinae, and Darininae are represented in the Southwestern Atlantic. The subfamily Zenatinae Dall, 1895 was synonymized by Beu (2006) with Lutrariinae.

The most conspicuous morphological character that defines the mactrids is the inverted V-shaped cardinal tooth in the left valve, formed by two single cardinal teeth. In addition, the right valve's cardinal teeth are not fused and the anterior and posterior lateral teeth have, in general, only one cusp. The hinge formula of mactrids follows the nomenclature of Bernard and Munier Chalmas (Cox in Moore, 1969). In order to quantify the morphological variation of the group several authors studied different anatomical characters like ctenidia morphology, labial palps fusion, siphons, stomach and ultrastructure (i.e. Atkins 1937, Yonge 1948, Purchon 1960, Stasek 1963, Taylor 1973). Other authors have contributed to the knowledge of this group by describing and discussing many species worldwide, including South America (Dall 1894, 1897, 1901, Deshayes 1853, 1854, 1855, Philippi 1845, 1893, Reeve 1854, Smith 1881, 1905, 1914, 1915, among others).

A literature search yielded 22 available species names that were proposed from Southwestern Atlantic waters (Carcelles and Williamson 1951, Castellanos 1970, Rios 1994, 2009). The type specimens of those species were mainly deposited in European institutions. In the first half of the 20th Century, Lamy (1913, 1914, 1917, and 1925) studied the living species described by Lamarck in the collection at the Museum national d'Histoire naturelle, Paris. Later, Carcelles (1944) collated the first catalogue of the mollusks found in Puerto Quequén, Buenos Aires province, including six species of Mactridae. Some decades later Castellanos (1970), in a catalogue of mollusks from the Buenos Aires coast, added *Mactra petitii* d'Orbigny, 1846 in 1834–1847.

This work is part of an ongoing revision of the Southwestern Atlantic mactrids living in the Brazilian, Argentine and Magellan zoological provinces.

MATERIALS AND METHODS

Specimens were collected from La Paloma and Montevideo, in Uruguay; Rio de la Plata estuary, San Clemente del Tuyú, Mar Chiquita, Mar del Plata, Miramar, Puerto Quequén, San Blas and the mouth of the Rio Negro river area, in Argentina (Fig. 1). The material was collected, using a shrimp trawl of 2.5 cm pore diameter, in the subtidal zone. All living samples were fixed in 10% formalin and then transferred to 70% ethanol. The specimens were dissected and observed with a stereomicroscope. Digital photographs of the shells were made with a Nikon D100 and a micro lens Nikkor 60 mm. The hinge was described with the method developed by Bernard and Munier Chalmas (according to Cox in Moore, 1969) where

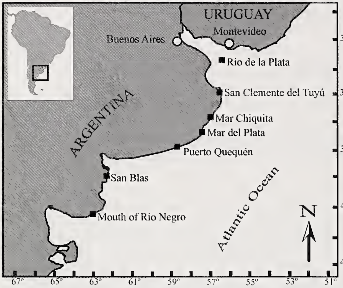

Figure 1. Map of the study area showing the sampled localities.

Arabic numbers are used to designate the cardinal teeth and roman numbers for the laterals. This method shows that all teeth in a right valve have odd numbers and all teeth in a left valve even numbers. The ultrastructure of the valves was analyzed in radial section. Siphons, ctenidia and labial palps were critical-point dried and then observed with a scanning electronic microscope (SEM) Phillips XL 30. The suprageneric classification used here follows Carter *et al.* (2011). The complete diagnosis of each valid species is summarized in the Table 1.

Type material of all nominal species and additional specimens from the following institutions were studied: Museo de la Plata (MLP); Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN-In), Buenos Aires; Museu Oceanográfico "Prof. E. C. Rios" (FURG), Rio Grande; Museu de Zoologia da Universidade de São Paulo (MZUSP), São Paulo; Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ), Rio de Janeiro; Museo Nacional de Historia Natural y Antropología (MNHNM), Montevideo; Museo Nacional de Historia Natural (MNHNC), Santiago; Academy of Natural Sciences, Philadelphia (ANSP); American Museum of Natural History (AMNH), New York; United States National Museum, Smithsonian Institution (USNM), Washington D.C.; Natural History Museum, London (NHMUK); Museum national d'Histoire naturelle (MNHN), Paris; Rijksmuseum van Natuurlijke Histoire (RMNH), Leiden; Zoologisk Museum (ZMUC), Copenhague, and Museum für Naturkunde (ZMB), Berlin.

RESULTS

SYSTEMATICS

Class Bivalvia Linnaeus, 1758
Subclass Autobranchia Grobben, 1894
Order Cardiida Férussac, 1822 in 1821–1822
Superfamily Mactroidea Lamarck, 1809
Family Mactridae Lamarck, 1809.

Genus *Mactra* Linnaeus, 1767

Trigonella Da Costa, 1778: 196 (objective synonym, by subsequent designation Winckworth, 1926: 106).

Deikea Mayer, 1872: 498 (type species, *Mactra gallensis* Mayer, 1867: 43; by subsequent designation Keen, 1969: 595).

Colorimactra Iredale, 1929: 268 (type species, *Mactra queenslandica* E. A. Smith, 1914: 148; by original designation).

Telemactra Iredale, 1929: 268 (type species, *Mactra obesa* Deshayes in Reeve, 1854: pl. 5, species 19; by original designation).

Type species:

Cardium stultorum Linnaeus, 1758: 681, by subsequent designation, Fleming 1818: 309.

Diagnosis:

Shell trigonal to oval, subequilateral, inflated; lunule and escutcheon well developed. External ligament separated from internal ligament by a shelly ridge. Pallial sinus shallow to deep and oval; lateral teeth elongated, similar in shape and size.

Distribution:

Atlantic coast of the American continent, Mediterranean Sea, United Kingdom coast, Atlantic and Indian coast of Africa, and coast of Australia and New Zealand.

Mactra guidoi Signorelli and Scarabino, 2010
(Figs. 2A–C)

This species was recently described. It is here illustrated with the only purpose to keep together the species of the same geographic area.

Distribution:

From Guarapari, Espírito Santo in Brazil to Valdés Peninsula in Argentina (Signorelli and Scarabino, 2010).

Remarks:

Mactra guidoi was confused for many years by authors with *Mactra patagonica* d'Orbigny, 1846(Figs. 2D–F) which was collected by d'Orbigny from Quaternary deposits in Río Negro province. The material deposited in the NHMUK clearly differs from the specimens of *Mactra guidoi* (Figs. 2A–C). Aguirre (1994) cited this divergence and maintained unresolved the nomenclatorial issue. Finally, after dealing with this misunderstanding back to Ihering (1907), a complete

Table 1. Comparative diagnostic characters of valid species living in Northern Argentina and Uruguay.

Species	*Mactra guidoi*	*Mactra isabelleana*	*Mactra marplatensis*	*Mactra petitii*	*Mactrella janeiroensis*	*Raeta plicatella*	*Darina solenoides*
Shell morphology	Oval, elongated, ventral edge sinuous	Trigonal, inflated escutcheon not defined	Oval, elongated, escutcheon well defined	Oval, fragile, porcelanous	Subtrigonal, fragile, escutcheon defined by a shelly line	Trigonal to ovoid, fragile, white, pellucid	Elliptical, fragile, thin, inequilateral, laterally compressed
maximum size (length) recorded	45 mm	65 mm	40 mm	45 mm	55 mm	75 mm	74 mm
Pallial sinus	Rounded and shallow, V-shaped	shallow, V shaped	Short	Shallow and high, U-shaped	Deep, U-shaped	Deep and high, U-shaped	Deep U-shaped
Hinge plate	Smooth lateral teeth	Short lateral teeth	Lateral teeth different in length	Lateral teeth fragile, resilifer poorly developed	Ventral lateral teeth stronger than dorsal ones	Concentrated in cardinal area, laterals rudimentary	Weak lateral teeth very close to cardinals
Posterior gap	Absent	Absent	Absent	Absent	Small posterior gap	Posterior gap	Anterior and posterior gap
External surface	Smooth	Smooth	Smooth	Smooth	Concentric ridges in the umbonal area	With concentric wrinkles	Smooth

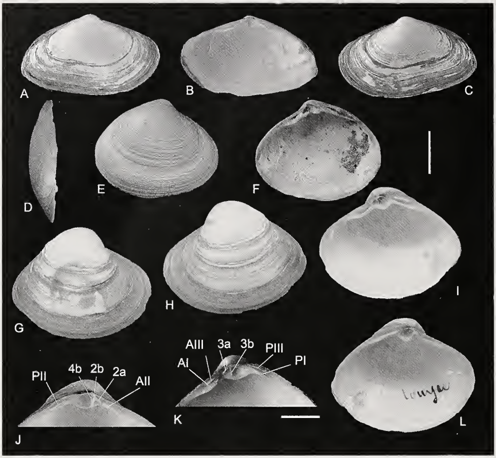

Figure 2. **A–C,** *Mactra guidoi,* Holotype MNHNM 15526; **A–B,** right valve, external and internal view; **C,** left valve, external view; **D–F,** *Mactra patagonica* Holotype NHMUK 1854.12.4.668; **D,** left valve, dorsal view; **E–F,** right valve, external and internal view; **G–L,** *Mactra isabelleana*; **G–J,** Lectotype NHMUK 1854.12.4.667/1, scale bar =1 cm; **K–L,** hinge detail of cardinal and lateral dentition, scale bar =1 cm. Arabic numbers are used to designate cardinal teeth and Roman numbers lateral teeth (Cox in Moore 1969).

revision of material of *Mactra patagonica* and *Mactra guidoi* from several institutions allowed us to establish that the first one was only recorded as a fossil form from Quaternary deposits and the second as recent shells from Buenos Aires and Uruguay (Signorelli and Scarabino, 2010).

Mactra isabelleana d'Orbigny, 1846

(Figs. 2G–L, 3A–J)

Mactra isabelleana d'Orbigny, 1846 in 1834–1847: 509, pl. 77, figs. 25–26. Weinkauff, 1884: 115; Smith, 1885: 58; Lamy, 1917: 194. Carcelles, 1944: 283, pl. 10, fig. 84; Barattini and Ureta 1961: 168, pl. 44, figs. a–b; Figueiras, 1961: 17; Parodiz, 1962: 36, 42–160; Richard and Craig, 1963: 137, pl. 2, figs. 7–8; Rios, 1966: 33; 1970: 194, pl. 57; 1975: 235, pl.74, fig. 1125; 1985: 242, pl. 86, fig. 1207; 1994: 265, pl. 90 fig. 1289; 2009: 535, fig. 1484; Cauquoin, 1967: 223; Figueiras and Sicardi, 1969: 365, pl. 4, fig. 53; Castellanos, 1970: 237, pl. 21, figs. 9–10; Closs and Forti Esteves, 1971: 32, pl. 3, figs. 7 a–b; Scarabino, 1977: 210, pl. 9, fig. 5; Forti Esteves, 1984: figs. 10 a–b; Aguirre, 1989: 169; 1991, 165; 1992: 39; 1994: 138, pl. 1, figs. 1–9, pl. 2, fig. 1–17; Scarabino, 2003: 245; Scarabino *et al.*, 2006: 157–169.

Trigonella isabellina d'Orbigny [sic]. Adams and Adams, 1853–1858: 376.

Mulinea isabelliana d'Orbigny [sic]. Conrad, 1868: 31.

Description:

Shell. Trigonal with irregular growth lines on the external surface, covered almost entirely by a yellow to brownish and dehiscent periostracum, anterior and posterior dorsal margins softly curved and descended to rounded ends; ventral margin also curved. Prosogyrous umbones present, not in contact. Rudimentary lunule and escutcheon not defined by shelly line. External ligament poorly developed, placed in a nymph oriented to the posterior side of the umbones. Internal ligament trigonal (Fig. 3C), located in a ventrally developed resilifer, both separated by a shelly ridge. Interior white; hinge with two anterior lateral teeth (AI and AIII) in the right valve; two not fused cardinal teeth (3a and 3b) placed dorsally to the resilifer and two posterior lateral teeth (PI and PIII); left valve with one anterior (AII) and one posterior (PII) lateral tooth; inverted V-shaped cardinal tooth composed by two single cardinals (2a and 2b), the 2b flanked by accessory lamella (4b) (Figs. 2K–L); left laterals with internal pustules well developed observed also in juveniles (Figs. 3H–I). External ligament poorly developed in juveniles (Fig. 3J). Ultrastructure of the shell with two layers, the inner with a cross-lamellar complex and the outer with simple cross lamellar structure (Figs. 3A–B). Juveniles with a protoconch very evident and a rudimentary hinge in both valves (Figs. 3D–G). Mantle cavity organs. Digestive system with mouth, short esophagus, stomach type V of Purchon (1960), connected to a long crystalline style on the posterior ventral margin, completely separated from the intestine and located into a sac (Fig. 4C), immersed in the digestive diverticula; intestine long, coiled and positioned surrounding the visceral mass; in the posterior

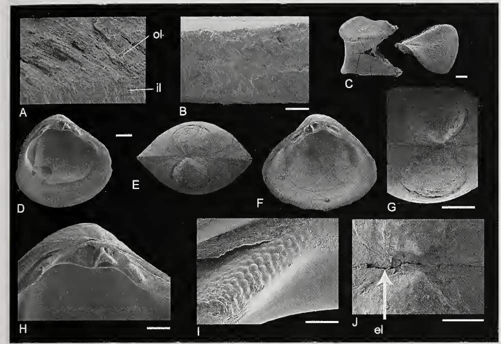

Figure 3. *Mactra isabelleana,* **A–B**, shell ultrastructure, scale bar 100 µm; **C**, SEM picture of external ligament, scale bar = 5 mm; **D–G**, SEM picture of juveniles showing the protoconch, scale bars, **D–F** = 2 mm, **G** = 1 mm; **H–I**, internal pustules present in lateral teeth, scale bar **H** = 500 µm, **I** = 20 µm; **J**, SEM picture of the rudimentary external ligament, scale bar = 100 µm. Abbreviations, **el**, external ligament; **il**, inner layer; **ol**, outer layer.

side of the body, intestine ascending to the pericardial cavity where it goes through the ventricle (Fig. 4D) and finally comes out through the anus, placed on the ventral side of the posterior adductor muscle in the base of the exhalant siphon. Siphons type C of Yonge (1948, 1957), completely fused with outer ring of sensory tentacles, but without categorization; both siphons distinguished, the exhalant smaller than the inhalant; mantle folds united with the periostracal groove (Figs. 4A–B). Ctenidia type C1 (Atkins 1937) fused in the posterior side of the body surrounding it laterally; each ctenidia formed by two demibranch. The inner demibranch with a food groove in the ventral margin. Labial palps trigonal, elongated with the inner surface plicated and the outer smooth, fused to the ctenidia with a type 3 of Stasek category (1963) (Fig. 4E).

Type Material:

Aguirre (1994) designated a lectotype (NHMUK 1854.12.4.667/1) and two paralectotypes (NHMUK 1854. 12.4.667/2, 3).

Type locality:

Montevideo, Uruguay. Additional specimens were sampled by Candé, A. Isabelle and by d'Orbigny themselves in the mouth of the Río de la Plata, Maldonado Bay in Uruguay and Cabo San Antonio, Argentina.

Other material examined: *Brazil.* Rio de Janeiro (RJ) (MZUSP 14678); Cabo Frio, RJ (MZUSP 80817); São Tomé, RJ (MUZSP 35091); São Paulo (SP) (FURG 32866; MNHNM 7350; MZUSP 22363, 34757); Iguape, SP (MZUSP 79653); São Sebastião, SP (MZUSP 81120); Matinhos, Paraná (PR) (MZUSP 22294); Santa Catarina (FURG 32807, 47886; MZUSP 77476, 40 m); Rio Grande do Sul (RS) (ANSP 225473, 249096; FURG 4353, 9860, 16901, 16994, 18914, 22239, 22390, 23074, 23458, 23684; MNRJ 1474, 8966; MZUSP 662, 16140, 45013); Albardão, RS (FURG 32353); Cassino, RS (AMNH 293596; FURG 1329, 5962, 7851, 11888, 40182, 43041; MZUSP 77475, 77477; NHMUK 1958-4-10-64). *Uruguay.* Chui (FURG 32673); Rocha (MNHNM 4584, 7354; USNM 335793, 359272); Cabo Polonio (USNM 359220); La Paloma (FURG 32576; MACN 15168, 15395, 15864, 17552; MNHNM 2400, 4282, 7339; MNRJ 1227; MZUSP 22366; USNM 362723); Flores (USNM 364200); Punta del Este (MNHNM 7334, 7346, 7353; NHMUK 1956-8-4-88-93); Maldonado (AMNH, 191876; ANSP 70491, 70497, 285624; MNHNM 2541, 3396, 7343, 7344; MZUSP 13058, 13059; USNM 151659, 180884, 180886, 343542); Piriápolis (FURG 8477); Montevideo (ANSP 312403, 350044; FURG 1591; MZUSP 8063,11559, 22364; USNM 122497, 331310, 331467, 334426, 347544, 355718, 359257, 363192, 363767, 380783; MACN 977, 6783-1, 8732, 9771-1, 11080, 29683; MLP 2367, 2461, 9387; MNHNM 2386, 2544, 4397, 7337, 7347, 7352; NHMUK 91-4-13-100, 4 valves with periostracum); Canelones (ANSP 221268, 314950; MNHNM 7342; MZUSP 22365; USNM 335794); Lobos (USNM 359258); Nueva Palmira (MACN 11351; USNM 331309). *Argentina.* Río de la Plata (USNM 96144, 424473); La Plata (MZUSP 13060, 13061); Punta Indio (MLP 3888); Mar del Plata (AMNH 191877; FURG 172; MACN 10306, 10772, 11580, 11964, 14152, 16578; USNM 346833); Miramar (NHMUK 1937-11-9-2); Necochea (MLP 3895); Puerto Quequén (MACN 14158); Bahía Blanca (MACN 6620-3); San Blas (MACN 20400); Río Negro (MLP 1859); San Antonio Oeste (FURG 17217).

Distribution:

From Cabo Frio, Rio de Janeiro state, Brazil to San Antonio Oeste, Río Negro province, Argentina.

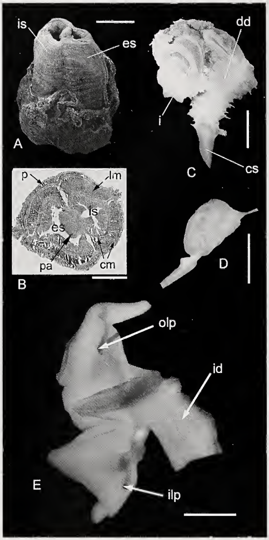

Figure 4. *Mactra isabelleana*, **A**, SEM picture of the siphons, scale bar = 1 mm; **B**, histological cross section of the siphons, scale bar = 500 μm; **C**, external view of digestive system, scale bar = 1cm; **D**, heart and hind gut, scale bar = 5 mm; **E**, inner demibranch associated to the labial palps, scale bar = 5 mm. Abbreviations, **cm**, circular muscle; **cs**, crystalline style; **dd**, digestive diverticula; **es**, exhalant siphon; **i** = intestine; **id**, inner demibranch; **ilp**, inner labial palp; **is**, inhalant siphon; **lm**, longitudinal muscle; **olp**, outer labial palp; **p**, periostracum; **pa**, papillae.

Remarks:

Mactra isabelleana is one of the dominant bivalves in muddy and sandy bottoms in the Río de la Plata estuary (Giberto *et al.* 2004). After a review of the quaternary species of *Mactra* from Buenos Aires Province Aguirre (1994) concluded that *Mactra isabelleana* is a "polytypic" species that includes the morphotypes *Mactra petitii* d'Orbigny, 1846 and *Mactra marplatensis* Doello-Jurado, 1949. However, after the analysis of the type material of the three nominal taxa, significant morphological differences were observed. Within this context, *Mactra isabelleana* is a valid species living in the Buenos Aires coast. It differs from *Mactra marplatensis* by the presence of a shell more trigonal and less elongated; a shorter pallial sinus, stronger hinge with lateral teeth shorter and mainly by a posterodorsal area not defined by a shelly ridge. *Mactra petitii*, a valid and common species from southern coast of Brazil, differs from *Mactra isabelleana* by the presence of a more fragile and less inflated shell. Other mactrids studied previously, such as *Mactra stultorum* (Linnaeus, 1758), *Spisula solidissima* (Dillwyn, 1817) and *S. polynyma* (Stimpson, 1860), present ctenidia with a type C (2) (Kellog, 1915).

Mactra marplatensis Doello-Jurado, 1949
(Figs. 5A–J)

Mactra marplatensis Carcelles, 1944, 283 (*nomen nudum*)
Mactra marplatensis Doello-Jurado, 1949: 4; Parodiz, 1962: 42–43; Rios, 1966: 33; 1970: 195; 1975: 235, pl.74, fig. 1126; 1985: 243, pl. 86, fig. 1208; 1994: 265, pl. 90, fig. 1290; Figueiras and Sicardi, 1969: 366, pl.4, fig. 55; Castellanos, 1970, 234, pl. 21, fig. 11–13.

Description:

Shell. Oval, elongated, equilateral, strong, with the external surface smooth covered by a brownish periostracum usually absent in the umbonal area; umbones subcentral and prosogyrous. Anterior end rounded ascending to the umbo straightly; ventral margin convex; posterior end with a more defined angle; escutcheon defined by a shelly ridge revealed by periostracal folds; lunule wide but poorly defined. Interior white, with a deep pallial sinus, U-shaped; adductor muscle scars similar and lenticular. Right hinge with two lateral teeth, different in length, being the AI more elongated than the AIII; resilifer small and rounded; posterior lateral teeth more elongated than the anterior, similar in size and shape; cardinal teeth not fused, with the anterior short but strong, oriented obliquely to the anterior side of the shell and the posterior more fragile and placed almost vertically. Left hinge with one anterior and one posterior lateral teeth similar in size and fragile; cardinal teeth fused making the typical inverted V-shaped tooth flanked by the accessory lamella (Figs. 5F–G). Internal ligament in a resilifer, separated from the external ligament by a fragile shelly ridge; external ligament rudimentary located behind the umbones in the dorsal area. Ultrastructure of the shell with same structure as the species previously analyzed (Figs. 5H–J).

Type Material:

Holotype (MACN-In 10307) articulated specimen with periostracum. Six paratypes (MACN-In 10307-1).

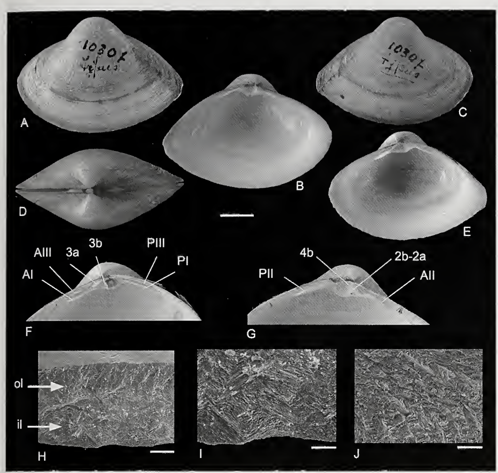

Figure 5. *Mactra marplatensis*, **A–E**, Holotype (MACN 10307), scale bar 1 cm; **F–G**, hinge detail, scale bar =1 cm; **H–J**, shell ultrastructure, **H**, SEM picture of radial cross section, scale bar 50 µm, **I**, detail of inner layer with CCL (complex cross-lamellar), **J**, detail of outer layer with CL (cross-lamellar) structure, scale bar = 20 µm. Abbreviations, **il**, inner layer; **ol**, outer layer. Arabic numbers are used to designate cardinal teeth and Roman numbers lateral teeth (Cox in Moore 1969).

Type Locality:

Mar del Plata, Argentina, in 30 m depth.

Other material examined:

Brazil. Recreio dos Bandeirantes, RJ (MNRJ 1473); Ilha Grande, RJ (MZUSP 22344); Rio de Janeiro (FURG 6602, 10165, 28175, 31903, 34398; MZUSP 22345, 22346, 44249); São Paulo (FURG 47019); São Sebastião, SP (FURG 43212); Ubatuba, SP (MZUSP 83652); Santa Catarina (FURG 42891); Cassino, RS (FURG 34355). *Uruguay.* Chui (MNHNM 8460); Playa del Barco, Rocha (MACN 14787); Fortaleza Santa Teresa, Rocha (MNHNM 8459); La Paloma (MNHNM 2387, 2408, 3117, 7336, 7351); Punta del Este (MNHNM 1088). *Argentina.* Río de la Plata estuary (MACN 23502, collected in 1938, 23 m, 4 valves); Mar Chiquita (MACN 21204); Mar del Plata (MACN 8812, 10307 (Holotype); MNHNM 7349); Miramar (MLP 1461); Puerto Quequén (MACN 13036).

Distribution:

From Rio de Janeiro, Brazil to Puerto Quequén, Buenos Aires province, Argentina.

Remarks:

Mactra marplatensis was first mentioned by Carcelles (1944) in a catalogue of marine mollusks from Puerto Quequén without a formal description, hence it is a *nomen nudum*. Its description was published posthumously by Doello-Jurado (1949).

Mactra petitii d'Orbigny, 1846
(Figs. 6A–G)

Mactra petitii d'Orbigny, 1846: 509, pl. 72, fig. 23–24; Martinez and Saez, 1869: 13, pl. 3, fig. 9; Ihering, 1907: 320; Lamy, 1917: 342; Cauquoin, 1967: 226; Castellanos, 1970, 234, pl. 21, fig. 4–6; Rios, 1970: 196, pl. 57; 1975: 236, pl. 75, fig. 1131; 1985: 243, pl. 86, fig. 1212; 1994: 265, pl. 90, fig. 1294; 2009: 536, fig. 1488; Altena 1971: 52; Aguirre, 1994: 141, pl. 1, figs. 5A–b.

Mactra symmetrica Deshayes in Reeve, 1854: pl. 16, fig. 84; Deshayes, 1854: 17; Dall, 1891: 44.

Trigonella petitii Conrad, 1868: 39.

Mactra scalpellum Dall, 1891: 44. (not Deshayes in Reeve, 1854).

Mactra richmondi Dall, 1894: 26.

Description:

Shell. trigonal to oval, inequilateral, elongate, maximum size up to 45 mm, relatively fragile; external surface mostly smooth; concentric ornamentation only present in the dorsal area at both sides of the umbones. Posterior end rounded; posterodorsal area or escutcheon well-defined from the umbones to the posterior end, ventral margin and anterior end rounded without sharp angles; small and poorly inflated umbones at ~middle of the shell length. Interior white with a short but high U-shaped pallial sinus. Hinge formula similar to *Mactra marplatensis*, but with the resilifer distinctly less ventrally developed; lateral teeth fragile and elongated, cardinal teeth symmetrically oriented in relation to the longitudinal axis, very close to the umbones (Fig. 6C); external ligament separated from internal ligament by a shelly ridge.

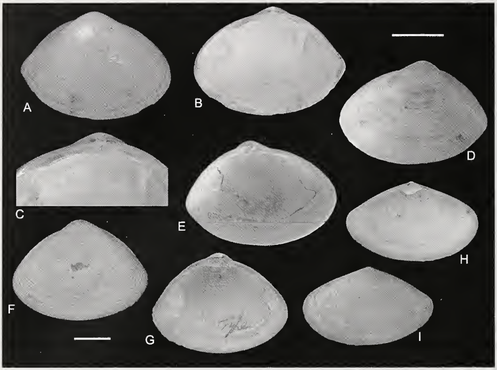

Figure 6. **A–C**, *Mactra petitii* Holotype NHMUK 1854-12-4-665; **D-E**, *Mactra richmondi* Holotype USNM 124774; **F–G**, *Mactra symmetrica* Holotype (NHMUK Cuming coll.); **H–I**: *Mactra scallpelum* Syntype (NHMUK unnumbered), scale bar = 1 cm.

Type Material:

[*Mactra petitii*] NHMUK 1854-12-4-665, holotype from Rio de Janeiro, Brazil; [*Mactra symmetrica*] NHMUK (unnumbered), holotype from Cuming collection, erroneously recorded from New Caledonia; [*Mactra richmondi*] USNM 124774, holotype collected in Greytown, Nicaragua.

Type Locality:

Rio de Janeiro, Brazil.

Other material examined:

Central America. Jamaica (AMNH 87614); Panama channel (AMNH 163694). *Brazil.* Rio Grande do Norte (ANSP 300090, 300476); Alagoas (AL) (FURG 11142, 29274); Caravelas, BA (MZUSP 22303); Espírito Santo (ES) (FURG 30430; MZUSP 15741); Guarapari, ES (MZUSP 15737, 22305, 22306, 22307, 22308); Vitória, ES (MZUSP 22304); Niterói, RJ (MNRJ 4081); Cabo Frio, RJ (USNM 426258); Flamengo, RJ (MZUSP 42752); Rio de Janeiro (AMNH 129246; FURG 8032, 16017, 28171, 46655; MNRJ 4579; MZUSP 19514, 21827, 22331, 55692; USNM 359314); Angra dos Reis, RJ (MZUSP 22315, 22329, 63182, 63239); Mangaratiba, RJ (MNRJ 1560); Ilha Grande, RJ (MNRJ 1476; MZUSP 22310, 22311, 22312, 22313, 22314, 22317, 22318, 22319, 22320, 22321, 22322, 22323, 22324, 22325, 22326, 22327, 22328, 22330; FURG 14258); Recreio dos Bandeirantes, RJ (MNRJ 1592, 2114); São Sebastião, SP (MZUSP 14178, 14246; MNHNM 2399); Santos, SP (FURG 11809, 43206, 43238; MNHNM 2389, 2399, 2402, 7348, 8669, 8698; MZUSP 22259, 22335, 22336, 22337, 22339, 78251); Ubatuba, SP (MZUSP 22260, 22332, 22333, 22334, 53953, 82477, 82536; MNHNM 2404); Barra de Guaratiba, RJ (MNRJ 1595); Matinhos, PR (MZUSP 22340); Barra do Sul, Santa Catarina (SC) (MZUSP 19374); Camboriú, SC (MZUSP 26347); Ilha de São Francisco, SC (MZUSP 13034); Santa Catarina (FURG 47741, 31679, MNRJ 376, MZUSP 22341); Albardão, RS (FURG 16906); Rio Grande do Sul (FURG 18044, 22902, 22970, 23042, 23058, 26785; MZUSP 70488). *Uruguay.* Rocha (MZUSP 660); Puerto La Paloma (MACN 29997); Maldonado (FURG 16214). *Argentina.* Necochea (MLP 9380); Monte Hermoso (MZUSP 13056).

Distribution:

From Jamaica to Monte Hermoso in Buenos Aires province, Argentina.

Remarks:

The type specimen of *Mactra petitii* (Figs. 6A–C) was collected by M. Cléry, and later sent to d'Orbigny by Petit de la Saussaye. *Mactra symmetrica* Deshayes in Reeve, 1854 and *Mactra richmondi* Dall, 1894 (Figs. 6D–G) are confirmed synonyms. The first one was erroneously described from New Caledonia, a colony of France, at that time. None of the catalogues on marine mollusks from Oceania (Smith 1914, Kershaw 1958, Lamprell and Whitehead 1990; Kilburn and Hylleberg 1998, Taylor and Glover 2004) mentioned *Mactra symmetrica. Mactra coquimbana* was illustrated by Philippi (1887) and proposed as a synonym of *Mactra petitii* by Ihering (1907). The illustration of *Mactra coquimbana* (Philippi, 1887: 244, pl. 30, fig. 2) from Neogene deposits from Coquimbo, Chile is rudimentary and does not allow confirmation of its taxonomic position. Finally, *Mactra scalpellum* Deshayes in Reeve, 1854 described from the New Zealand coast was erroneously mentioned by Dall (1891) for Brazil. The type material of this valid Indo-Pacific species (Figs. 6H–I) clearly differs from *Mactra petitii.*

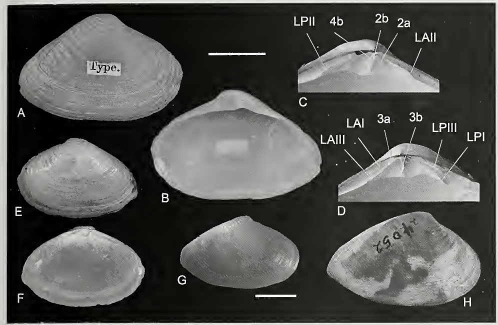

Figure 7. **A–B**, *Mactrella janeiroensis*, Syntype NHMUK 1915.4.18.489, scale bar 1 cm; **C–D**, *Mactrella janeiroensis*, hinge detail; **E–F**, *Mactra surinamensis* Holotype (RMNH unnumbered); **G**, original illustration of *Micromactra miskito* (Petuch, 1998); **H**, *Mactra californica maracaibensis* Holotype PRI 24052, scale bar = 1 cm. Arabic numbers are used to designate cardinal teeth and Roman numbers lateral teeth (Cox in Moore 1969).

Genus *Mactrella* Gray, 1853

Papyrina Mörch, 1853: 4, in part, objective synonym Keen, 1969: 598.

Micromactra Dall, 1894: 40 (type species, *Mactra californica* Conrad, 1837: 240; by monotypy).

Type species:

Mactra striatula Linnaeus, 1767: 1125; by monotypy.

Diagnosis:

Shell trigonal to oval, inequilateral, with concentric lines in the umbonal area; posterior end defined by shelly line weakly visible; hinge with anterior lateral teeth with two cusps.

Distribution:

Europe, Pacific coast of North America, western Atlantic from Caribbean Sea to Argentina.

Mactrella janeiroensis (E. A. Smith, 1915)
new combination
(Figs. 7A–G, 8A–H)

Mactra (*Mactrinula*) *janeiroensis* E. A. Smith, 1915: 102, pl. 4, fig. 20.

Mactra janeiroensis E. A. Smith, 1915. Doello-Jurado in Carcelles, 1944: 284, pl. 10, fig. 83; Barattini and Ureta, 1961: 169; Figueiras, 1962: 62; Cauquoin, 1967: 224; Figueiras and Sicardi, 1969: 367, pl.4, fig. 56; Rios, 1969: 9; 1970: 195, pl. 57; 1975: 236, pl.75 fig. 1128; 1985: 243, pl. 86, fig. 1213; 1994: 266, pl. 91, fig. 1295; 2009: 536, fig. 1489; Castellanos, 1970: 236, pl. 21, figs. 7–8.

Mactra surinamensis Altena, 1968: 172, figs. 152; 1971: 52, fig. 18.

Mactra miskito Petuch, 1998: 40, fig. 12–13.

Description:

Shell. subtrigonal, elongated, thin, fragile, inequilateral, with concentric ornamentation weaker along the ventral margin; subequilateral, umbones prosogyrous and weakly inflated; anterior and posterior ends rounded, brownish periostracum eroded in umbonal area; lunule not defined by a shelly line but recognized; escutcheon well developed and limited by a line or a soft keel. Interior white and pellucid with a deep and U-shaped pallial sinus. Right hinge like mactrids discussed above, with two cardinal teeth (3a and 3b) not fused. Anterior lateral teeth of different sizes, the ventral one longer; two posterior lateral teeth (PI and PIII) similar in size; left valve with inverted V-shaped cardinal teeth (2a and 2b) flanked in the posterior side by the accessory lamella (4b); one anterior and one posterior lateral tooth complete the hinge (Figs. 7C–D). Resilifer well developed ventrally. Ultrastructure of the shell like other mactrids analyzed (Fig. 8H). Mantle cavity organs. siphons completely fused covered by the periostracum, inhalant with three sizes of tentacles in the apertural ring (Figs. 8B, D, E and G); Exhalant siphon with two rows of tentacles of uniform size (Fig. 8C); morphology of the ctenidia and labial palps similar to those of previous species (Fig. 8F).

Type material: [*Mactrella janeiroensis*] NHMUK Reg. 1915.4.18.489, 6 syntypes. The illustrated specimen in the original description had the following dimensions: Length: 31 mm, height: 20 mm, width: 12 mm; [*Mactra surinamensis*] RMNH (unnumbered), holotype from Suriname and four paratypes collected in Coppename, Suriname; [*Mactra miskito*] holotype (single right valve, CMNH 47364), length 30 mm, width 19 mm; 5 paratypes (single valves, CMNH 47352).

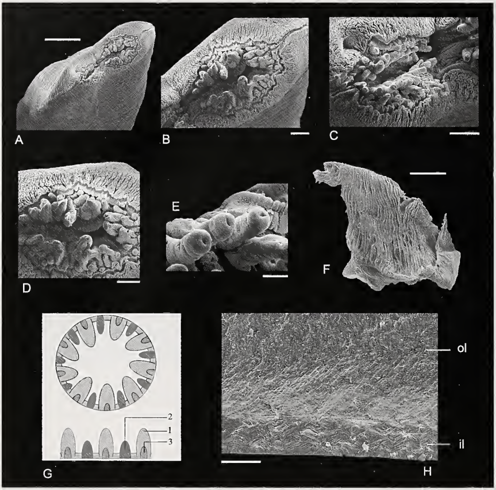

Figure 8. *Mactrella janeiroensis*, **A–E**, SEM picture of siphons; **A**, general aspect; **B**, inhalant siphon; **C**, exhalant siphon; **D**, tentacles of inhalant siphon; **E**; Detail of tentacles of inhalant siphon, scale bar = 200 µm for all figures except E = 20 µm; **F**, outer labial palp, scale bar = 1 mm; **G**, scheme of inhalant siphon with the three size of tentacles; **H**, shell ultrastructure, scale bar = 100 µm. Abbreviations, **il**, inner layer, **ol**, outer layer.

Type locality:

Rio de Janeiro, Brazil, collected in sandy bottoms at 73 m. depth (station 42 of "Terra Nova" Antarctic Expedition, May 1913).

Other material examined:

Venezuela. Venezuela (USNM 381914). *Brazil*. Maceió, AL (FURG 37524); Bahia (FURG 29337); Espírito Santo (FURG 1290); Guarapari, ES (FURG 15541); Rio de Janeiro (MNHNM 7370); Ilha Grande, RJ (MZUSP 22353, 22355); Cabo Frio, RJ (MZUSP 19373); São Paulo (FURG 12224, 43240, 46969, 47702; MNHNM 2406, 7995; MZUSP 13043, 82277); Ubatuba, SP (MZUSP 22348, 22349, 22350, 22351, 22395, 22396, 77960, 82479, 83137; MNHNM 8730); Santos, SP (MZUSP 22352, 44998, 45199, 45207); Bertioga, SP (MZUSP 79750); Cananéia, SP (MNHNM 2405; MZUSP 26169, 83451); Santa Catarina (MZUSP 15777, FURG 47724, 46847); Santa Catarina lagoon (MNHNM 3617); Porto Belo, SC (MZUSP 36318, FURG 42752); Rio Grande do Sul (FURG 13173, 14009, 21921, 22608, 22893); Albardão, RS (FURG 17006, 32927); Cassino, RS (FURG 49). *Uruguay*. Cabo Polonio (FURG 15154); La Paloma: (MNHNM 2403, 4410). *Argentina*. Mar del Plata (MACN 8809, 23376); Pinamar (85 km to east: MACN 24249, 23411); Quequén (MACN 2309); Gulf of San Matías (MLP 4124); Gulf of San José, Chubut (MACN 37944).

Distribution:

From Santa Rita, Venezuela to Villarino beach, San José Gulf, Argentina.

Remarks:

The placement of *Mactrella janeiroensis* into this genus is based on the similarities between *Mactra californica* Conrad, Genotype of *Micromactra*, and *Mactra striatula* Linnaeus, Genotype of *Mactrella*. The full description and comparison of the genotypes of *Mactrella*, *Micromactra*, *Mactrinula* and *Mactrellona*, is currently in process of publication. *Mactrella janeiroensis* and *Raeta plicatella* are the South American mactrids with largest distribution in the Western Atlantic. Both species occur from the Caribbean Sea to the North Patagonian gulfs in Argentina. The distribution differs from other mactrids (i.e. *Trinitasia iheringi* (Dall, 1897), *Mactrotoma fragilis* (Gmelin, 1791) and *Mactrellona alata* (Spengler, 1802) that never extend beyond Santa Catarina, Brazil. *Mactrella janeiroensis* has been usually cited in South American catalogues (Carcelles 1944, Barattini and Ureta 1961, Cauquoin 1967, Rios 1969, 1975, 1994, 2009, Figueiras and Sicardi 1969, Castellanos 1970). *Mactra* (*Micromactra*) *surinamensis* Altena, 1968 (Figs. 7E–F), described from the coast of Suriname, is a confirmed synonym. It is characterized by the diagnostic concentric ornamentation and the escutcheon; however the specimens described by Altena were broken. In the internal side of the shell, the hinge and the pallial sinus are equal to those observed in the types of *Mactrella janeiroensis*. Finally, other nominal species that must be considered as synonym is *Micromactra miskito* Petuch, 1998 (Fig. 7G) described from the coast of Nicaragua. The Miocene Venezuelan *Mactra* (*Mactrotoma*) *californica maracaibensis* Hodson and Hodson,

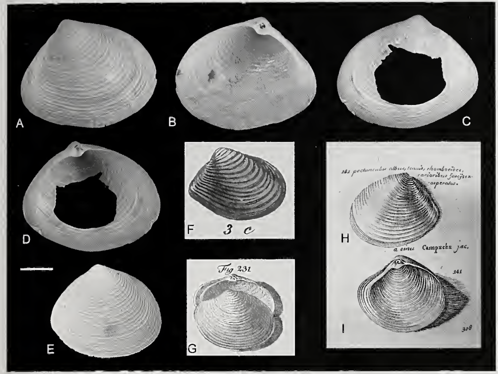
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Figure 9. A–D, *Raeta plicatella*, Syntypes (MNHN); **E**, *Lavignon papyracea* d'Orbigny, 1846 (NHMUK 1854.12.4.686); **F**, *Mactra campechensis*, original illustration from Wood and Hanley (1828); **G**, *Mactra papyracea*, illustration from Chemnitz (1782); **H–I**, *Mactra campechensis* illustration from Lister (1770); scale bar = 1 cm.

1931 is similar to *Mactrella janeiroensis*. However, the shell morphology of the types of *maracaibensis* (Holotype PRI 24052 and Paratype PRI 24061 collected in the Miocene locality of Río Coquiza, about 2 km, north and 6 km East of Miranda, Venezuela) differs in the posterodorsal end, being more elongated, than that of the living one (Fig. 7H), and therefore, is not considered a valid species.

Subfamily Kymatoxinae Stenzel *et al.*, 1957.
Genus *Raeta* Gray, 1853.
Lovellia Mayer, 1867: 253 (objective synonym).

Type species:

Mactra campechensis Gray, 1825: 134 (= *Lutraria plicatella* Lamarck, 1818 in 1815–1822: 470, by monotypy).

Diagnosis:

Shell trigonal, fragile, pellucid, completely ornamented in the external surface by concentric wrinkles, compressed in the posterior area, anterior and posterior end rounded, lateral teeth rudimentary and close to the cardinals.

Distribution:

Europe, Pacific coast of Central and South America, Western Atlantic and Asia.

Raeta plicatella (Lamarck, 1818)

(Figs. 9A–F, H–I, 10A–G)

Mactra papyracea Chemnitz, 1782 (*nomen nudum*), 6: pl.23, fig. 231.
Mactra papyracea Chemnitz, 1782. Gmelin, 1791: 3257.
Lutraria plicatella Lamarck, 1818: 470; Deshayes, 1835: 93; Hanley, 1842 in 1842–1856: 27; Lamy, 1913: 347.
Lutraria canaliculata Say, 1822: 311; Conrad, 1831: 46, pl. 10, fig. 1; De Kay, 1843: 232, pl. 31, fig. 298.
Mactra campechensis Gray, 1825: 134; Wood and Hanley, 1828: pl. 1, fig. 3.
Lutraria campechensis. Gray, 1837: 375.
Lavignon papyracea Gmelin. d'Orbigny, 1846 in 1834–1847: 527.
Raeta campechensis Gray, 1853: 43.
Mactra canaliculata. Reeve, 1854: pl. 21, fig. 122.
Raeta canaliculata. Adams and Adams, 1853–1858: 386, pl. 102, fig. 44a; Chenu 1862: 62, fig. 251; Carpenter, 1863: 368; Conrad, 1868: 41.
Lovellia canaliculata. Mayer, 1867: 37.
Raeta perspicua Hutton, 1873: 65.
Labiosa (*Raeta*) *canaliculata.* Dall, 1895: 212; 1894: 28; Vanatta, 1903, 55: 757; Mauri, 1920: 137.
Anatina canaliculata Say, 1822: Perry, 1940: 83, pl. 18, fig. 117.
Labiosa plicatella. Carcelles, 1944: 284, pl. 10, fig. 85; Barattini and Ureta, 1961: 170; Rios, 1966: 33; 1970: 197; Castellanos, 1970, 238, pl. 21, fig. 14–16; Figueiras and Broggi, 1972–1973: 221.
Raeta plicatella. Figueiras and Sicardi, 1969: 368, pl.4, fig. 57; Harry, 1969: 12, fig. 11–13; Altena, 1971: 54, pl. 5, fig. 4–6; Rios, 1975: 237, pl. 75, fig. 1136; 1985: 244, pl. 86, fig. 1217; 1994: 267, pl. 91, fig. 1300; 2009: 538, fig. 1494; Aguirre, 1989: 169 1991: 165; 1992: 39.

Description:

Shell. trigonal to ovoid, subequilateral, pellucid, inflated through the anterior portion, maximum size up to 75 mm length; external surface with diagnostic concentric strong wrinkles from umbones to ventral margin; anterodorsal and ventral margin rounded, posterior end rounded but more defined than the anterior; lunule and escutcheon not defined by a line or shelly ridge. Interior white with soft wrinkles that reveal the external ornamentation; deep pallial sinus with a length about 2/3 shell length; adductor muscle scar similar in size and shape, the anterior one more elongated dorso-ventrally.

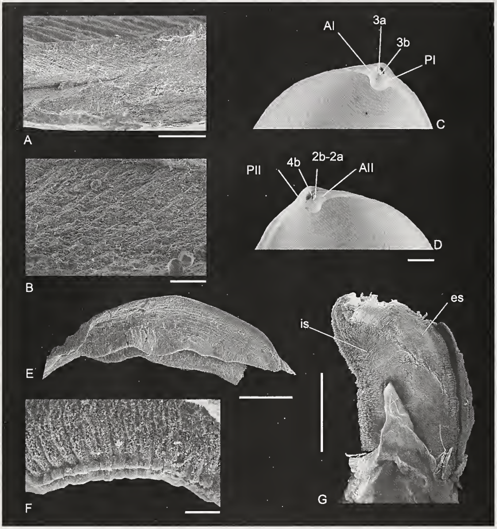

Figure 10. *Raeta plicatella*, **A–B**, shell ultrastructure, scale bar A = 200 µm, B = 20 µm; **C–D**, hinge detail of cardinal and lateral teeth, scale bar = 2 cm; **E–F**, SEM picture of ctenidia, scale bar = 1 mm; **F**, detail of food groove in the ventral edge, scale bar = 50 µm; **G**, SEM picture of siphons, scale bar 2 mm. Arabic numbers are used to designate cardinal teeth and Roman numbers lateral teeth (Cox in Moore 1969).

Right hinge with only one anterior and posterior lateral tooth and two cardinal teeth not fused; laterals very short and close to the cardinals; left hinge with the inverted V-shaped cardinal teeth closely flanked by the accessory lamella; one anterior and one posterior lateral tooth; resilifer very well developed (Figs. 10C–D). Ultrastructure of the shell with the same layers that other mactrids (Figs. 10A–B). MANTLE CAVITY ORGANS. Siphons completely fused, the inhalant bigger than the exhalant (Fig. 10G). Two demibranchs at both sides of the body dorsally fused to the mantle with an elongated morphology. The inner demibranch bigger than the outer with a food grove in the ventral edge (Figs. 10E–F); labial palps notoriously developed, trigonal, placed surrounding the mouth and fused dorsally with the visceral mass.

Type material: [*Raeta plicatella*] MNHN (unnumbered), two syntypes; [*Lutraria campechensis*] type material not found, not deposited in the NHMUK; [*L. canaliculata*] type material not housed in the ANSP; [*R. perspicua*] NMNZ 0321, holotype registered erroneously from Bay of Islands, New Zealand.

Type Locality: "*l´Indian Ocean*" is mentioned in the original description.

Other material examined: *United States of America*: Virginia (VA) (AMNH 310600); Hog Island, VA (USNM 27583); Fort Macon, North Carolina (NC) (USNM 407354); Beaufort, NC (MNRJ 1293; USNM 24585, 27587, 278117, 467425, 488236, 595610); Myrtle Beach, South Carolina (SC) (USNM 54360, 631769, 8077190); Charleston Bay, SC (USNM 27586, 103783, 462419, 462431); Folly Beach, SC (USNM 420530); Jekyll Island, Georgia (GA) (AMNH 309266; USNM 462417, 462432); St. Simons, GA (USNM 27582); St. Catherine Island, GA (AMNH 171652, 183311, 271933, 271988, 272059); St. Simons Island, GA (USNM 535380); Florida (AMNH 34007,113735, 115344, 172210, 248029; FURG 40204; USNM 43189, 54358, 54368, 60784, , 92113, 198442, 487332, 765899, 781697); St. Augustine, FL (USNM 462433); Mouth of Saint Johns River, FL (USNM 46847); Hillsboro inlet, FL (USNM 87640); Key West, FL (AMNH 2725749); Cape Romano, FL (USNM 150365, 533753); Marco Island, FL (AMNH 248031, 311464; USNM 614164); Bonita Springs, FL (AMNH 292618);Sanibel, FL (FURG 8584; MNRJ 1292; MZUSP 46885; NHMUK 2206; USNM 168853; AMNH 16108, 87075, 103278, 115346, 120440, 139099, 157821, 1 specimen, 179331, 181427, collected in 1972, 2 specimens, 203190, 203196, 243762, 265146, collected in 1973, 266856, 272575, 292617, 294018, 309280, 310617); Captiva, FL (AMNH 100057); Lee county, FL (USNM 53687); Mullet Key, FL (USNM 27585); Boca Ciega, FL (FURG 4574); Panama City, FL (USNM 18353, 466243, 518351); Alabama (AMNH 210004, 210308); Louisiana (USNM 177932, 189171); Texas (AMNH 177884; MNRJ 1291; USNM 126184, 6037; FURG 28056); Bolivar Point, TX (USNM 465337). Matagorda, TX (USNM 134379);

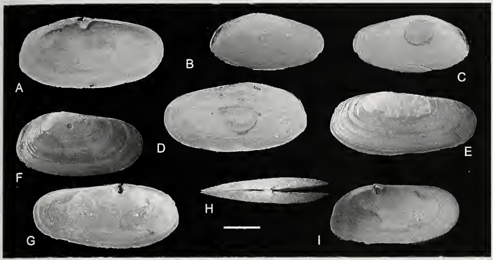

Figure 11. **A–D**, *Darina solenoides*, Syntypes NHMUK 1837.12.1.879/884; **E–I**, *Lutraria tenuis*; **F**, Holotype ZMB 112740; **E, G–I**, Paratypes ZMB 112741, scale bar = 1 cm.

Brazil. Maceió, AL (FURG 37482; MNRJ 4578); Espírito Santo (FURG 10203); Rio de Janeiro (MZUSP 45145); Búzios, RJ (FURG 20970); Ilha Grande, RJ (MZUSP 22421); São Paulo (MZUSP 13088, 22430, 45015, 45018, 45019, 45163, 45174, 77492, 80969, 84041); Guarujá, SP (MZUSP 15855); Ubatuba, SP (FURG 10558; MZUSP 22422, 22423, 22424, 22425, 22426, 22427, 22428, 22429, 22434, 22454, 45022, 45187, 46485, 82431); Santos, SP (USNM 359298; MZUSP 45189); Peruíbe, SP (MZUSP 22431, 45021); Bertioga, SP (MZUSP 22433); Iguape, SP (MZUSP 13089, 22432); Praia do Saí, PR (MZUSP 22435); Paranaguá, PR (FURG 5179); Porto Belo, SC (FURG 18121, 42978, 46584; MZUSP 36311); Santa Catarina (USNM 122498; FURG 50250; MZUSP 309); Camboriú, SC (FURG 31684); Florianópolis, SC (MZUSP 56993); Itapema, SC (MZUSP 16122); Cassino, RS (FURG 3449, 4155, 66). *Uruguay.* Chui (FURG 17548); La Coronilla, Uruguay (FURG 32644); La Paloma, Rocha (USNM 361685, 426418; AMNH 177972; FURG 9003, 9514, 19022, 24223); Rocha, Uruguay (USNM 362984). *Argentina.* Buenos Aires (FURG 46311); Villa Gessell (MLP 5375); Puerto Quequén, Argentina (USNM 381677); Monte Hermoso (MLP 2042).

Distribution:

From Hog Island, Virginia State, United States of America to Monte Hermoso, Argentina.

Remarks:

Lutraria canaliculata Say, 1822, is a synonym. Unfortunately the type material of this taxon was not found at the ANSP collection where most of Say's material is deposited. In the original description Say did not illustrate this species. Later, this name is mentioned and illustrated in several publications (i. e. Conrad 1831, De Kay 1843, Adams and Adams 1853–1858, Reeve 1854, Chenu 1862, Perry 1940). After an analysis of the shell morphology of all those illustrations we conclude that *L. canaliculata* Say must be considered a synonym of *Raeta plicatella. Mactra campechensis* Gray, 1825 (Fig. 9F), based on the illustration of Lister (1770) from one specimen (Fig. 9H–I) collected in Campeche, Mexico, coincides with the morphology of *R. plicatella*, and therefore, is also considered a synonym.

The revision of the type material of *Raeta perspicua* Hutton, 1873 revealed it as synonymous with Lamarck's species. Hutton described it erroneously from New Zealand as previously mentioned Harry (1969). In the prologue of Hutton's publication it is mentioned that several specimens were donated by private collectors commonly labeled incorrectly. In addition, there is no mention of this species in several mollusk catalogues from Australia and New Zealand (Smith 1914, Kershaw 1958, Lamprell and Whitehead 1990, Kilburn and Hylleberg 1998, Taylor and Glover 2004).

Mactra papyracea Gmelin, 1791 is mentioned as a possible older name (Lamarck, 1818). This species was described originally by Chemnitz (1782, vol. 6, pl. 23, fig. 231), whose work was rejected by nomenclatorial reasons (ICZN 1944, 1954). The type material was not found in European malacological collections. If this synonymy is confirmed the valid name must be changed to *Raeta papyracea* (Gmelin, 1791). Unfortunately Chemnitz's illustration is rudimentary (Fig. 9G). Only d'Orbigny (1846 in 1834–1847) mentioned the name introduced by Chemnitz into the genus *Lavignon* d'Orbigny 1845 in 1834–1847 (Fig. 9E). The specimens from d'Orbigny collection coincide with *R. plicatella* Lamarck.

Subfamily Darininae Signorelli in Carter *et al.* 2011.
Genus *Darina* Gray, 1853.

Type Species:

Erycina solenoides King, 1832: 335, by monotypy.

Diagnosis:

Shell fragile, thin, elongated, compressed laterally, yellowish periostracum; siphonal gap in both ends; small external ligament, internal ligament well-developed placed in a chondrophore; teeth weak, located in the cardinal area; pallial sinus deep.

Distribution:

From Bahía Blanca in Argentina to Puerto Mont in Chile.

Darina solenoides (King, 1832) (Figs. 11A–I)

The full morphological characterization of this species was carried out in the revision of Magellanic mactrids (Signorelli and Pastorino 2011).

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Comparative morphology of *Leucozonia* from Brazil (Neogastropoda: Buccinoidea: Fasciolariidae)

Diogo Ribeiro Couto and Alexandre Dias Pimenta

Setor de Malacologia, Departamento de Invertebrados, Museu Nacional/Universidade Federal do Rio de Janeiro, Quinta da Boa Vista, São Cristóvão, CEP 20940-040, Rio de Janeiro, Rio de Janeiro, Brazil.

Correspondence, Diogo Ribeiro Couto: diogoaut@gmail.com

Abstract: The morphology of the fasciolariid gastropods *Leucozonia nassa* (Gmelin, 1791), *L. ocellata* (Gmelin, 1791) and *L. ponderosa* Vermeij and Snyder, 1998 from Brazil is described. Examination of several shells from the oceanic island of Trindade, off southeastern Brazil confirmed the conchological differences between *L. ponderosa* and *L. nassa*. In addition, the first occurrence of *L. ponderosa* is documented from the Fernando de Noronha Archipelago, off northeastern Brazil. The anatomy of *L. ponderosa* is shown to be identical to *L. nassa*, except for minor radular features; however, the male genital system was not examined because of the lack of male specimens of *L. ponderosa* available for study. *Leucozonia ocellata* differs from *L. nassa* and *L. ponderosa* in having a proportionally smaller seminal receptacle and bursa copulatrix, a rhynchostome with lip-like margins, a curved anterior esophagus, and a cement gland with a posteriorly folded branch. Anatomic characters of the species studied here agree with the previous descriptions of fasciolariid anatomy.

Key words: anatomy, fasciolariid, western Atlantic, Peristerniinae, *Leucozonia nassa*

Fasciolariids are important members of the molluscan fauna in shallow coastal waters at depths of up to 70 m (Petuch 1987), especially on sandy substrates. Rosenberg (2009) reported 92 species from the western Atlantic, distributed among 17 genera.

The genus *Leucozonia* Gray, 1847 is common in shallow coastal waters and is represented in Brazil by *Leucozonia nassa* (Gmelin, 1791), *L. ocellata* (Gmelin, 1791), and *L. ponderosa* Vermeij and Snyder, 1998. *Leucozonia ponderosa* is endemic to Trindade Island, an oceanic island off the coast of the state of Espírito Santo in southeastern Brazil. *Leucozonia nassa* and *L. ocellata* have wider geographical ranges on the continental shelf from the northeastern to the southern coast of Brazil, as well as the oceanic islands of Trindade and Fernando de Noronha (Leal 1991, Rios 2009). They undergo intracapsular or lecithotrophic development (Leal 1991), as do other fasciolariids.

The most common species, *Leucozonia nassa*, has a marked geographical differentiation, leading to the occurrence of several synonyms, and at least three distinct morphs based solely on shell characters, which were considered by Vermeij (1997) as three different species, *L. nassa*, *L. leucozonalis* (Lamarck, 1822), and *L. brasiliana* (d'Orbigny, 1841). In contrast, Vermeij and Snyder (2002) argued that these characters alone may be insufficient to allow separation of species. The endemic species from Trindade Island, *L. ponderosa* was considered by Rios (2009) as a synonym of *L. nassa*.

Knowledge of the anatomy of the Fasciolariidae is somewhat sparse. Marcus and Marcus (1962) presented a fine anatomical study of *Leucozonia nassa* from Brazil. Although these authors provided a thorough characterization of the species from the state of São Paulo coast in southeastern Brazil, including histological sections, they did not illustrate several features such as the head-foot, pallial cavity, and male reproductive and digestive systems. More recently, Kosyan *et al.* (2009) studied the comparative anatomy of seven fasciolariid species. Typically, fasciolariids have an orange-red head-foot, and lack a caecum in the stomach and an ingesting gland in the pallial oviduct; additionally, as in buccinids, melongenids, and nassariids, they lack accessory salivary glands and an anal gland (Harasewych 1998). Kantor (2003) distinguished the Fasciolariidae by their distinct stomach morphology with low folds on the inner wall, transverse striations on the longitudinal fold and absence of a posterior mixing area, and stated that the superfamily Buccinoidea can be differentiated based on stomach characters. Fraussen *et al.* (2007) reported that a combination of anatomical characters is diagnostic for the Fasciolariidae: the multicuspid lateral teeth and a small rachidian tooth, single or paired proboscis retractor muscle, salivary ducts embedded in the esophagus wall and the characteristic stomach morphology. Kosyan *et al.* (2009) confirmed the proboscis retractor muscles as characteristic for the fasciolariids.

Here, we compare the anatomy of the three species of the genus *Leucozonia* from Brazil.

MATERIALS AND METHODS

The study was based on material deposited in the malacological collection of Museu Nacional da Universidade Federal do Rio de Janeiro (MNRJ) and Museu de Zoologia da

Universidade de São Paulo (MZSP). The specimens were collected during fieldwork in Abrolhos and Fernando de Noronha Archipelagos, and coasts of the states of Bahia, Rio de Janeiro and Pernambuco state. More recent collections were made by the authors in Cabo Frio, Rio de Janeiro state.

The specimens were fixed in ethanol 70%. Shells were measured with a caliper and photographs of individuals were taken with a Canon PSS X100 IS digital camera. Anatomical dissections were made using a NIKON SMZ1500 stereoscope microscope. All drawings were made using a camera lucida.

The following material was selected for dissections: 12 specimens out of 200 analyzed for *Leucozonia nassa* (MNRJ 10925, MNRJ 10993, MZSP 69496, MNRJ 15161, MNRJ 14485); seven specimens out of 46 analyzed for *L. ocellata* (MNRJ 10735, MNRJ 12963); three female specimens of *L. ponderosa* were available for dissection (MNRJ 5220, MNRJ 14607). In the material examined, the number within brackets indicates the number of shells or specimens (shells + soft parts) in each lot.

Radulae were manually extracted and prepared by immersion in KOH, followed by ultrasonic cleaning in distilled water for SEM photography using a Jeol JSM-6390LV at "Centro de Microscopia Eletrônica", in Departamento de Invertebrados, Museu Nacional/ Universidade Federal do Rio de Janeiro (UFRJ).

Anatomical terminology is based on Simone (2000) and Simone *et al.* (2009); conchological descriptions were based on Vermeij and Snyder (2002).

RESULTS

Family Fasciolariidae Gray, 1853
Subfamily Peristerniinae Tryon, 1880
Genus *Leucozonia* Gray, 1847
Type species: *Murex nassa* Gmelin, 1791 by original designation.

Leucozonia nassa (Gmelin, 1791)
(Figs. 1–4)

Murex nassa Gmelin, 1791: 3551.
Voluta fuscata Gmelin, 1791: 3465.
Fusus cingulatus Röding, 1798: 119.
Murex rubicunda Perry, 1811: pl. 54, fig. 2.
Fasciolaria cingulifera Lamarck, 1816: pl. 429, figs. 1a–b.
Turbinella cingulifera (Lamarck, 1816): Reeve (1847, pl. 3, fig. 17).
Turbinella brasiliana d'Orbigny, 1841: 449, pl. 77, fig. 17.
Turbinella knorrii Deshayes, 1843: Reeve (1847: pl. 10, fig. 52).
Turbinella angularis Reeve, 1847: pl. 10, fig. 49.
Turbinella (*Leucozonia*) *fuscata* (Gmelin, 1791): Mörch (1852: 100).
Turbinella (*Leucozonia*) *nassa* (Gmelin, 1791): Mörch (1852: 100).
Turbinella (*Leucozonia*) *knorrii* (Deshays, 1843): Mörch (1852: 10).
Leucozonia cingulifera (Lamarck, 1816): Lange de Morretes (1949: 99).
Peristernia inculta Gould, 1860: 328.
Leucozonia brasiliana (d'Orbigny, 1841): Lange de Morretes (1949: 99).
Latirus riiseana (Kobelt, 1874): Dall (1885: 134).
Leucozonia riiseana (Kobelt, 1874): Dall (1885: 134).
Latirus (*Leucozonia*) *cingulifera* (Lamarck, 1816): Dall (1889: 112).
Latirus cinguliferus (Lamarck, 1816): Baker (1891: 50).
Leucozonia nassa (Gmelin, 1791): Weisbord (1962: 360–361, pl. 32, figs. 5–8); Abbott (1954: 240–241, pl. 11d); Warmke and Abbott (1961: 120–121, pl. 22m); Marcus and Marcus (1962: 11–24, pl. 1–2); Abbott (1968: 146, fig. 4); Rios (1970: 95, pl. 26); Abbott (1974: 226, fig. 2485); Rios (1975: 103, pl. 29, fig. 433); Vokes and Vokes (1983: 27, pl. 16, fig. 10); Bandel (1984: 143, pl. 17, fig. 5); Rios (1985: 107, pl. 37, fig. 472); Eston *et al.* (1986: 43); Matthews-Cascon *et al.* (1989: 362–364); Leal (1991: 157, 392–393, 417, pl. 20, figs. B–C); Rios (1994: 133–134, pl. 42, fig. 576); Díaz and Puyana (1994: 197, fig. 759); Vermeij and Snyder (2002: 25–29, figs. 1–14); Mallard and Robin (2005: pl. 56, 57); Rios (2009: 253–254).
Leucozonia nassa nassa (Gmelin, 1791): Abbott (1958: 78, pl. 2, fig. m).
Leucozonia nassa brasiliana (d'Orbigny, 1841): Abbott (1958: 79).
Leucozonia caribbeana Weisbord (1962: 361–362, pl. 32, figs. 9–10).
Leucozonia jacarusoi Petuch, 1987: A1–A2, pl. 29, figs. 4–5.

Type locality:
Unknown.

Types:
Unknown.

Distribution:

U.S.A.: Off North Carolina to Florida; Caribbean Islands: Bahamas; north coast of Cuba; south coasts of Hispaniola and Jamaica; eastern Caribbean from Puerto Rico to Trinidad and Barbados; Mexico: Veracruz, Campeche, and western Quintana Roo; Central-South America: coast of Costa Rica to Baía de Guaratuba, Santa Catarina state, Brazil; Brazilian

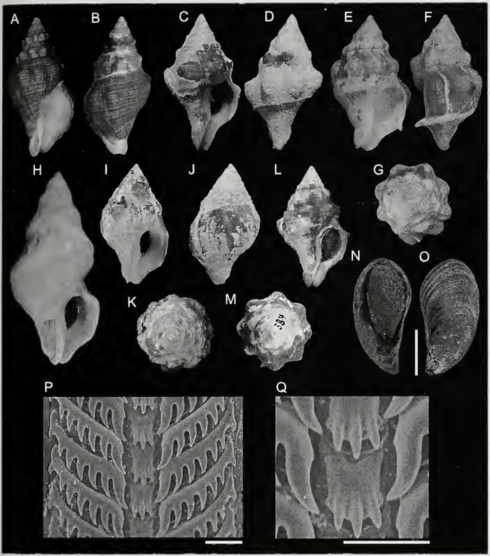

Figure 1. *Leucozonia nassa.* **A–M**, Shells; **A–B**, 55.5 mm (MNRJ 10993); **C–D**, 28.1 mm (MZSP 41814); **E–G**, 57.9 mm (MNRJ 14848); **H**, 69.9 mm (MNRJ 10710); **I–K**, 27.4 mm (MNRJ 14485); **L–M**, 36 mm (MNRJ 584); **N**, Operculum in inner view; **O**, Operculum in outer view; **P**, Radula in general view of median rows; **Q**, Detail of rachidian teeth of radula. Scale bars: 14–15: 3.0 mm; 16–17: 50 μm.

islands of Fernando de Noronha, Atol das Rocas, and Trindade (Vermeij and Snyder 2002).

Material examined:

U.S.A.: MNRJ HSL584, Marathon Key, Florida, U.S.A. da Mata leg. iii/1951 [2 shells]; Puerto Rico: MNRJ 9469, Michapel, Crash Boat Beach, Puerto Rico, col. 25/i/1964 [2 shells]; Brazil: Pernambuco state: MNRJ 10710, Formoso River, Tamandaré Bay, S. Ypiranga col. xii/1962 [7 specimens]; Atol das Rocas, off Pernambuco state: MNRJ 4281, J. H. Leal, G. W. Nunam, C. B. Castro, D. F. Moraes Jr col. ii–iii/1982 [18 shells]; Fernando de Noronha Archipelago, Pernambuco state: MNRJ 11182, Dois Irmãos Bank, 28m depth, P. M. S. Costa, R. S. Gomes col. 08/vii/1999 [1 shell]; Alagoas state: MNRJ 3161, Ponta Verde, Maceió, P. Sá Cardoso col. 1958 [7 shells]; Bahia state: MNRJ 15161, Conceição, Itaparica Island, D. R. Couto col. 4/i/2010 [10 specimens]; MNRJ 14848 Itapuã, Salvador, D. Mendonça col. xi/1964 [2 shells]; MNRJ 9345, Bainema, Boipeba, Eq Malacol. col. vii/1977 [17 specimens]. MNRJ 14019, São Tomé do Paripe, D. Campos col. vii/1973 [1 specimen]; MNRJ 14486, Itapuã, Salvador, D. R. Mendonça col. xi/1964 [1 specimen]; Abrolhos Archipelago, off Bahia state: MNRJ 14492, Station 1, Santa Bárbara Island, A. L. Castro, J. Becker, P. Jurberg col. ix/1969 [3 shells]; MNRJ 4877, Redonda Island, D. F. Mendonça Jr. col. 20/i/1984 [12 specimens]; Espírito Santo state: MZSP 41814, Guarapari, Coltro Leg. 2003 [14 specimens]; MZSP 69496, Escalvada Island, off Guarapari, under rocks at 12–20m, A. Bodart col. (from Femorale) i/2005 [2 specimens]; Trindade Island, off Espírito Santo state: MNRJ 14485, in tide pool 1m depth, F. Moraes col. 18/xi/2003 [1 specimen]; Rio de Janeiro, state: MNRJ 10925, Japonês Beach, Cabo Frio, A. D. Pimenta, P. M. S. Costa, J. B. Alvim, D. R. Couto col. 06/xi/2006 [131 specimens]; MNRJ 10993, Japonês Beach, Cabo Frio, A. D. Pimenta, P. M. S. Costa, J. B. Alvim, D. R. Couto col. 18/i/2007 [66 specimens].

Description:

Shell (Figs. 1A–M): Shell variable, elliptical and moderately fusiform, length to 69.6 mm, usually twice as long as wide; color chestnut to brown. Spire relatively high with angle varying from 55° to 75°, spire usually ~1/2 to 2/3 of total shell length. Protoconch small with two whorls, smooth; terminal varix low. Teleoconch with five to eight convex whorls, with two weak to strong angulations, one at shoulder and the other at central cord, sub-sutural ramp straight to concave, suture impressed; base of shell concave. Spiral sculpture variable, up to 27 cords, usually well marked at shoulder and central cords, and/or several secondary fine threads throughout the entire teleoconch. Axial sculpture of eight to 11 ribs generally forming elevated nodes more prominent on the teleoconch angulations, more so at

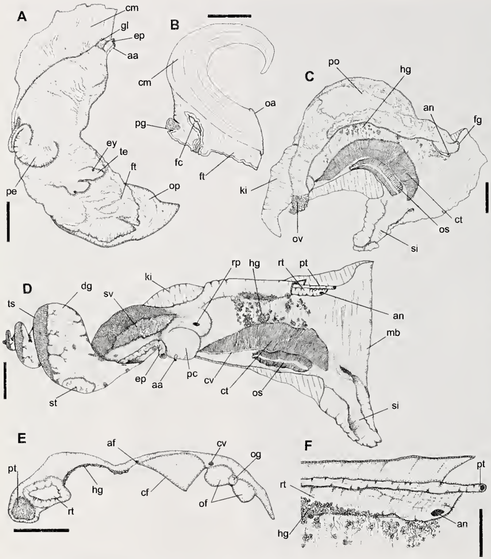

Figure 2. *Leucozonia nassa*. **A**, Head-foot mass in dorsal view, male; **B**, Longitudinal section of foot in median line, female; **C**, Pallial cavity roof, female; **D**, Visceral mass and pallial cavity roof in ventral view, male; **E**, Transversal section of pallial cavity roof in middle region of osphradium, male; **F**, Detail of prostate and rectum. Abbreviatons, **aa**, anterior aorta; **an**, anus; **af**, afferent branchial vessel; **cf**, ctenidium filament; **cm**, columellar muscle; **ct**, ctenidium; **cv**, ctenidial vein; **dg**, digestive gland; **ep**, posterior esophagus; **ey**, eye; **fc**, female cement gland; **fg**, female gonopore; **ft**, foot; **gl**, gland of Leiblein; **hg**, hypobranchial gland; **ki**, kidney; **mb**, mantle border; **oa**, opercular pad; **of**, osphradium filament; **og**, osphradium ganglia; **op**, operculum; **os**, osphradium; **ov**, ovary; **pc**, pericardium; **pe**, penis; **pg**, pedal gland furrow; **po**, pallial oviduct; **pt**, prostate; **rp**, renal aperture; **rt**, rectum; **si**, siphon; **st**, stomach; **sv**, seminal vesicle; **te**, cephalic tentacle; **ts**, testis. Scale bars, 2.0 mm.

shoulder; axial sculpture may be obsolete on later whorls. Aperture roughly elliptical to pyriform, height length about twice the width. Columella bearing three to five small folds in its center. Outer lip thin, marked internally by 12 to 14 beaded lirae, a distinct labral tooth at end of central cord may be present. Siphonal canal small, its length approximately 1/3 of total aperture height. Siphonal fasciole barely to highly distinct. Umbilical slit usually absent.

Head-foot (Figs. 1N–O, 2A–B): color dark red, head prominent, of medium size (width ~1/2 of adjacent head-foot width), with a pair of blunt and moderately long cephalic tentacles (length ~1/2 of anterior width of head), situated very close to each other, their bases lying side by side. Eyes dark, small and rounded situated in middle region of outer edge of the cephalic tentacles. Foot short and rounded, its anterior region bifid. Pedal gland as a shallow median anterior slit, with its anterior furrows extending along entire anterior edge of foot. Female cement gland opening at ~1/3 from anterior edge of foot, forming a somewhat elongate and relatively deep (of ~same foot thickness) sac, recurved anteriorly, of nearly smooth walls. Operculum corneous, ungulated (width ~1/2 of length), filling entire aperture; outer surface opaque, with anterior nucleus (Figs. 1N–O); inner surface with attachment scar elongated, elliptical, situated posteriorly, occupying ~2/3 of inner area. Columellar muscle thick, with ~1.5 whorls in length.

Pallial complex (Figs. 2C–F): pallial cavity wide, spanning about one whorl; mantle border simple, thickened. Siphon short (length ~1/2 of free portion of mantle edge), its margin smooth; right fold of siphon base extending into the pallial cavity, ending close to the anterior end of the ctenidium. Osphradium elongated and elliptical, slightly thinner posteriorly, its anterior margin rounded; length ~1/2 of that of the ctenidium; almost symmetrical longitudinally (its right side slightly wider); osphradium leaflets rounded, tall (height ~2/3 of ctenidial filament height at middle region of pallial cavity), almost equal in size. Ctenidium curved spanning ~1/3 of total pallial cavity area, its width ~1.5 the width of osphradium; posterior region acute, situated very close to pericardium; filaments triangular; ctenidial vein uniformly narrow along its length. Hypobranchial gland thin, located between gill and rectum, absent at posterior most area of pallial cavity. Rectum straight and elongated, fixed in place by a longitudinal membrane extending its whole length; rectum and prostate bound by a thin membrane. Anus small and

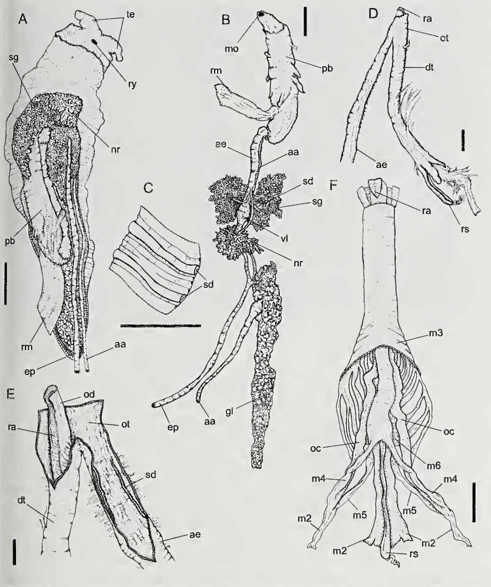

Figure 3. *Leucozonia nassa.* **A**, Head and haemocoel, in ventral view, foot and Columellar muscle removed, inner structures as in situ; **B**, Foregut extended, in ventral view; **C**, Longitudinal cut showing ventral inner wall of anterior esophagus anteriorly to valve of Leiblein; **D**. Buccal mass in lateral view; **E**, Buccal mass, in lateral view, anterior wall of esophagus and oral tube partially opened longitudinally; **F**, Odontophore in dorsal view. Abbreviations: **aa**, anterior aorta; **ae**, anterior esophagus; **dt**, odontophore tube; **ep**, posterior esophagus; **gl**, gland of Leiblein; **m2**, odontophore retractor muscle; **m3**, odontophore superficial circular muscle; **m4**, odontophore dorsal tensor muscle of radula; **m5**, odontophore auxiliary dorsal tensor muscle of radula; **m6**, odontophore horizontal muscle; **mo**, mouth; **nr**, nerve ring; **oc**, odontophore cartilage; **od**, odontophore; **ot**, oral tube; **pb**, proboscis; **ra**, radula; **rm**, proboscis retractor muscles; **rs**, radular sac; **ry**, rhynchostome; **sd**, salivary gland duct; **sg**, salivary gland; **te**, cephalic tentacle; **vl**, valve of Leiblein. Scale bars: 24–25: 3.0 mm; 26–29: 1.0 mm.

circular situated at 1/4 of pallial cavity length from mantle edge.

Digestive system (Figs. 1P–Q, 3A–F, 4A–C): Rhynchostome a small transverse slit, located between and below the cephalic tentacles. Proboscis straight, long (~2/3 of haemocoel length), with thick muscular walls; a single powerful proboscis retractor originates in the ventral posterior wall of the proboscis; laterally to the proboscis, a series of short muscles fibers connect to the inner walls of the haemocoel. Mouth small and circular. Odontophore long and slender (~proboscis length), formed by a pair of odontophore cartilages dorsally concave, fused anteriorly at ~40% of total cartilage length; a series of transverse muscle fibers connect the odontophore tube with the anterior esophagus, while a series of thin muscle fibers, the superficial circular muscles (m3) envelope odontophore as a whole except for the most posterior end; horizontal muscle (m6), originates on ventral surface of odontophore cartilages, except at posterior most region; a pair of odontophore retractor muscles (m2) originate from the posterior end of the odontophore cartilages, near to the radular sac, and are inserted in the posterior inner wall of proboscis; a second branch of m2, originates from the inner surface of the proboscis, near the origin of m2, enveloping the radular sac; pair of dorsal tensor muscles of radula (m4) originate from posterior end of odontophore, covering its dorsal surface; pair of auxiliary dorsal tensor muscles of radula (m5) originate from posterior end of the odontophore, covering its ventral surface. Radula (Figs. 1P–Q): radula long and thin; radular sac extending beyond the posterior end of odontophore; rachidian tooth straight, rectangular, its base with a concave outline and its cusped margin of convex outline, bearing three sharp cusps, the central one larger; lateral tooth wider then long, bearing six to seven centrally recurved cusps, the innermost being about twice as long as the others which have ~the same size, except for the outermost, slightly smaller; lateral margin acute, ending in a more external, last cusp; occurrence of a small denticle-like projection on inner margin of the innermost cusp. On one specimen, all of the left lateral teeth had an additional smaller cusp, while the right ones bore seven. Anterior esophagus moderately long and broad (~same length as proboscis), originating in the oral tube. Valve of

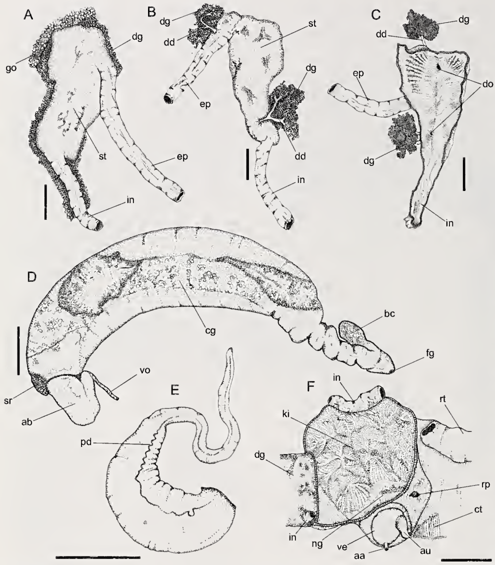

Figure 4. *Leucozonia nassa*. **A**, Stomach in dorsal view; **B**, Stomach in ventral view; **C**, Internal view of stomach, esophagus partially opened longitudinally; **D**, Pallial oviduct in ventral view; **E**, Penis; **F**, Renopericardial region in ventral view opened longitudinally, part of posterior region of pallial cavity also shown. Abbreviations: **aa**, anterior aorta; **ab**, albumen gland; **au**, auricle; **bc**, bursa copulatrix; **cg**, capsule gland; **ct**, ctenidium; **dd**, duct of digestive gland; **dg**, digestive gland; **do**, digestive opening; **ep**, posterior esophagus; **fg**, female gonopore; **go**, gonad; **in**, intestine; **ki**, kidney; **ng**, nephridial gland; **pd**, penis duct; **rp**, renal aperture; **rt**, rectum; **sr**, seminal receptacle; **st**, stomach; **ve**, ventricle; **vo**, visceral oviduct; Scale bars: 3.0 mm.

Leiblein pyriform, as an orange ring around the esophagus, ~1.5 the anterior esophagus width. Salivary glands just anterior to valve of Leiblein, forming a separate pair of branching and amorphous masses; free portion of salivary ducts short, extending to the esophagus anteriorly to the valve of Leiblein, where the ducts become merged with the esophagus wall, following anteriorly and opening in the esophagus lumen, immediately before the oral tube. Accessory salivary glands absent. Middle esophagus short; duct of gland of Leiblein short and narrow, situated posterior to the nerve ring. Gland of Leiblein brownish, long and narrow~ same length of posterior esophagus, its posterior end acute. Posterior esophagus longer than anterior. Posterior and middle esophagus narrower than anterior esophagus. Inner ventral wall of anterior esophagus with well marked longitudinal folds, the two laterals broader into which the salivary ducts are immersed; dorsal wall almost smooth; inner wall of posterior esophagus with very tiny folds. Stomach as a wide sac with thin walls bearing many internal folds. Digestive gland dark-brown, occupying all whorls of visceral mass, from apex to kidney/pericardium area, surrounding the stomach and emitting two narrow and branching ducts that discharge into the stomach near the esophagus and intestine apertures. Intestine as wide as esophagus, with longitudinal folds along inner wall.

Female Genital system (Figs. 2C, 4D): ovary intense red, occupying all whorls of visceral mass, surrounding apically the entire length of digestive gland and extending as far as the kidney. A thin visceral oviduct leads to a massive and ample cylindrical-shaped pallial oviduct, which occupies ~1/3 of total area of the pallial cavity roof, covering the rectum and part of the hypobranchial gland; albumen gland small (~1/10 of capsule gland length), curved halfway, becoming separated into a narrow proximal end and a large, thick distal end; capsule gland very large (~3/4 pallial oviduct length), external surface with distinct patterns of color and texture. A small seminal receptacle with a granular surface occurs dorsally between albumen and capsule glands. Dorsally, near the highly contracted and narrow terminal end of pallial oviduct, a saccular bursa copulatrix occurs, anteriorly to the small and elliptical gonopore.

Male Genital system (Figs. 2D, 4E): testis of similar position, shape and color of ovary. Visceral *vas deferens* running from testis and connecting to an elongated, intensely coiled seminal vesicle on mid-ventral region of last whorl of visceral mass; vas deferens narrow, simple, running along ventral wall of kidney, connecting to a thin and long tubular prostate, located along the right side of the roof of the pallial cavity, next to the rectum and of almost equal length. Penis

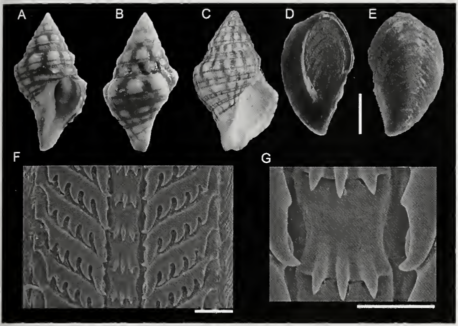

Figure 5. *Leucozonia ocellata.* **A–C,** Shells; **A–B,** 17 mm (MNRJ 10735); **C,** 35.9 mm (MNRJ 5357); **D,** Operculum in inner vies; **E,** Operculum in outer view; **F,** Radula in general view of median rows; **G,** Detail of rachidian teeth of radula. Scale bars: 39–40: 3.0 mm; 41–42: 50 μm.

elongated, broad at the base, sub-circular in section; becomes abruptly narrower at mid-length, terminating in a long and thin thread-like extension, reaching far back into the interior of pallial cavity, duct of penis very narrow, sinuous, more linear in its distal region, along its thread-like extension.

Circulatory and excretory systems (Fig. 4F): pericardium small, spanning ~1/3 of total renal cavity area; auricle tiny and pyriform, ventricle large and rounded, with thick walls. Kidney bearing ventral (primary) and dorsal (secondary) lamellar lobes similar in shape, lobes interdigitated corresponding to pycnonephridia according to Perrier (1889) and Ponder (1973); nephridial gland almost indistinct, situated on dorsal side of membrane between renal cavity and pericardium; renal aperture as a slit in the membrane between pallial and renal cavities, transverse to the roof of pallial cavity. Part of the intestine runs longitudinally on inner side of kidney, ventrally adhered to its membrane.

Leucozonia ocellata (Gmelin, 1791)
(Figs. 5–8)

Buccinum ocellatum Gmelin, 1791.

Turbinella ocellata (Gmelin, 1791): Mörch (1852: 100).

Turbinella nigella Mörch 1852: 100; Adams and Adams (1853: 154).

Latirus (*Leucozonia*) *ocellatus* (Gmelin, 1791): Dall (1889: 318).

Latirus ocellatus (Gmelin, 1791): Baker (1891: 50).

Fusus (*Leucozonia*) *ocellatus* (Gmelin, 1791): Coulon (1936: 128).

Leucozonia ocellata (Gmelin, 1791): Lange de Morretes (1949: 99); Weisbord (1962: 358–359, pl. 32, figs. 3–4); Warmke and Abbott (1961: 121, pl. 22k); Rios (1970: 95); Abbott (1974: 226, fig. 2484); Rios (1975: 103, pl. 29, fig. 434); Vokes and Vokes (1983: 16, pl. 16, fig. 12); Bandel (1984: 143, pl. 17, fig. 6); Rios (1985: 107, pl. 37, fig. 473); Matthews-Cascon *et al.* (1989: 362); Leal (1991: 157–158, 393); Rios (1994: 134, pl. 42, fig. 577); Vermeij (1997); Vermeij and Snyder (2002: 31–32, fig. 23–30); Mallard and Robin (2005: pl. 57); Rios (2009: 254).

Leucozonia occellata (Gmelin, 1791): Galindo (1977: 223) *error pro Leucozonia ocellata.*

Type locality:
Unknown.

Types:
Unknown.

Distribution:

U.S.A.: Key Largo, Florida; Bahamas; Caribbean Islands; Central-South America: Quintana Roo, Mexico to south to Rio de Janeiro, Brazil (Vermeij and Snyder, 2002).

Examined material:

Brazil: Fernando de Noronha archipelago, off Pernambuco state: MNRJ 11174, Rasa Island, P. M. S. Costa col. 10–11/vii/1999; MNRJ 11200, Buraco da Raquel, in tide pool in low tide, P. M. S. Costa col. 09/vii/1999 [1 shell]; Atol das Rocas, off Pernambuco state: MNRJ 4276, J. H. Leal, G. W. Nunam, C. B. Castro, D. F. Moraes Jr. col. ii–iii/1982 [10 shells]; Bahia state: MNRJ 5357, Itapoa beach, Salvador, P. Jurberg col. 11/vii/1980 [4 shells]; Abrolhos archipelago, off Bahia state: MNRJ 12963, station 1, SE of Santa Bárbara Island, A. L. Castro, J. Becker, P. Jurberg, A. Coelho col., ix/1968 [35 specimens]; MNRJ 10735, station 4, Santa Bárbara Island, A. L. Castro, J. Becker, P. Jurberg, A. Coelho col., ix/1969 [11 specimens]; Rio de Janeiro state: MNRJ 14223, L. R. Tostes col. iii/1975 [1 shell]; MNRJ 10736, Prainha Beach, Arraial do Cabo, A. L. Castro, J. Becker, P. Jurberg, E. A. Coelho col. ix/1969 [1 specimen].

Description:

Shell (Figs. 5A–C): Shell elliptical/biconic and moderately fusiform, maximum length 35.9 mm, ~twice as long as wide;

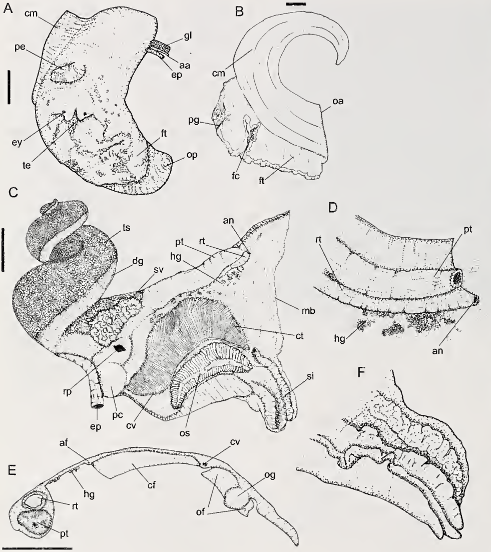

Figure 6. *Leucozonia ocellata*. **A,** Head-foot mass in dorsal view, male; **B,** Longitudinal section of foot in median line, female; **C,** Pallial cavity roof, male; **D,** Detail of prostate, rectum and anus; **E,** Transversal section of pallial cavity roof in middle region of osphradium, male; **F,** Detail of siphon. Abbreviatons: **aa**, anterior aorta; **af**, afferent branchial vessel; **an**, anus; **cf**, ctenidium filament; **cm**, columellar muscle; **ct**, ctenidium; **cv**, ctenidial vein; **dg**, digestive gland; **ep**, posterior esophagus; **ey**, eye; **fc**, female cement gland; **ft**, foot; **gl**, gland of Leiblein; **hg**, hypobranchial gland; **mb**, mantle border; **oa**, opercular pad; **of**, osphradium filament; **og**, osphradium ganglia; **op**, operculum; **os**, osphradium; **pc**, pericardium; **pe**, penis; **pg**, pedal gland furrow; **pt**, prostate; **rp**, renal aperture; **rt**, rectum; **si**, siphon; **sv**, seminal vesicle; **te**, cephalic tentacle; **ts**, testis. Scale bars: 1.0 mm.

color chestnut to brown, with whitish nodes. Spire high with angle 65° to 70°, spire length 1/2 to 2/3 of total shell length. Protoconch small, with 1.5 whorls, smooth; terminal varix low. Teleoconch with five to six convex whorls; sub-sutural ramp mildly concave, suture impressed; base of shell straight to slightly concave. Spiral sculpture of five to seven main cords and several fine cords throughout the teleoconch, less prominent at base. Axial sculpture of nine to 10 ribs forming elevated nodes at shoulder; nodes may be less prominent but 15 to 20 axial riblets are present. Aperture somewhat elliptical, height ~twice the width. Columella bearing three to four small folds in its center. Outer lip thin, marked internally by seven to 10 beaded lirae. Siphonal canal relatively small, its length ~half of total aperture height. Siphonal fasciole may be distinct. Umbilical slit absent.

Head-foot (Figs. 5D–E, 6A–B): color cream in preserved specimens; head prominent, of medium size (width ~1/2 of adjacent head-foot width), with a pair of short cephalic tentacles (length ~1/5 of anterior width of head), situated very close to each other, their bases lying side by side. Eyes as in *Leucozonia nassa*. Foot short and rounded posteriorly. Pedal gland as in *L. nassa*. Female cement gland opening at ~1/3 from anterior edge of foot, forming a somewhat elongate and relatively deep (of ~same foot thickness) sac, recurved anteriorly, of nearly smooth walls with a posterior folded branch. Operculum (Figs. 5D–E) as in *L. nassa* with attachment scar occupying ~1/2 of inner area. Columellar muscle as in *L. nassa*.

Pallial complex (Figs. 6C–F): pallial cavity wide, spanning ~3/4 of a whorl; mantle border as in *Leucozonia nassa*. Siphon short (length ~1/3 of free portion of mantle edge), its margin forming three distinct folds, the outermost smooth and the innermost highly contracted; right fold of siphon base extending into the pallial cavity, ending close to the anterior end of the ctenidium. Osphradium elongate and elliptical, slightly thinner anteriorly and posteriorly; length ~the same of that of the ctenidium; its right side wider; osphradium leaflets triangular with acute projections, tall (height ~same of ctenidial filament height at middle region of pallial cavity), its right leaflets larger. Ctenidium roughly curved, spanning ~1/3 of total pallial cavity area, its width twice that of osphradium; anterior and posterior regions acute, posterior end situated very close to pericardium; filaments as low triangles; ctenidial vein uniformly narrow along its length. Hypobranchial gland, rectum and anus as in *L. nassa*.

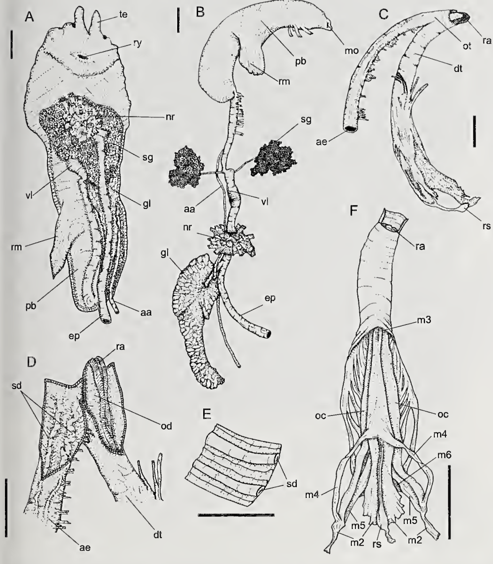

Figure 7. *Leucozonia ocellata.* **A**, Head and haemocoel, in ventral view, foot and Columellar muscle removed, inner structures as in situ; **B**, Foregut extended, in ventral view; **C**, Buccal mass in lateral view; **D**, Buccal mass, in lateral view, anterior wall of esophagus and oral tube partially opened longitudinally; **E**. Longitudinal cut showing ventral inner wall of anterior esophagus anteriorly to valve of Leiblein; **F**, Odontophore in dorsal view. Abbreviations: **aa**, anterior aorta; **ae**, anterior esophagus; **dt**, odontophore tube; **ep**, posterior esophagus; **gl**, gland of Leiblein; **m2**, odontophore retractor muscle; **m3**, odontophore superficial circular muscle; **m4**, odontophore dorsal tensor muscle of radula; **m5**, odontophore auxiliary dorsal tensor muscle of radula; **m6**, odontophore horizontal muscle; **mo**, mouth; **nr**, nerve ring; **oc**, odontophore cartilage; **od**, odontophore; **ot**, oral tube; **pb**, proboscis; **ra**, radula; **rm**, proboscis retractor muscles; **rs**, radular sac; **ry**, rhynchostome; **sd**, salivary gland duct; **sg**, salivary gland; **te**, cephalic tentacle; **vl**, valve of Leiblein. Scale bars: 1.0 mm.

Digestive system (Figs. 5F–G, 7A–F, 8A–C): Rhynchostome forming a small transverse slit emarginated by thin lip-like folds, located between and below the cephalic tentacles. Proboscis and mouth as in *Leucozonia nassa*. Odontophore as in *L. nassa*, but fused anteriorly only ~30% of total cartilage length. Radula (Figs. 5F–G): radula long and thin; radular sac extending beyond the posterior end of odontophore; rachidian tooth straight, rectangular, its base with a concave outline and its cusped margin with convex outline, bearing three sharp cusps of about the same size; lateral tooth wider then long, bearing five centrally recurved cusps, the innermost being about twice as long as the others which have approximately the same size, except for the outermost, slightly smaller; lateral margin acute; occurrence of a denticle-like projection on inner margin of innermost cusp. Anterior esophagus moderately long (~same length as proboscis), bearing a small curve immediately before the valve of Leiblein, originating in the oral tube. Valve of Leiblein pyriform, as an orange ring around the esophagus, ~same width of anterior esophagus. Remaining features of digestive system as in *L. nassa*.

Female genital system (Fig. 8D): ovary color cream in fixed specimens, occupying the same position as in *Leucozonia nassa*. A visceral oviduct leaves to a massive and ample cylindrical-shaped pallial oviduct, which occupies ~1/3 of total area of the pallial cavity roof, covering the rectum and part of the hypobranchial gland; albumen gland small (~1/5 of capsule gland), curved halfway, becoming separated into a narrow proximal end and a large, thick distal end; capsule gland very large (~3/4 of pallial oviduct length), external surface with distinct patterns of color and texture. A seminal receptacle with a granular surface occurs dorsally between albumen and capsule glands. Dorsally, near the narrow terminal end of pallial oviduct, a saccular bursa copulatrix occurs, anteriorly to the small and elliptical gonopore.

Male genital system (Figs. 6C, 8E–F): Visceral and pallial portion of male genital system as in *Leucozonia nassa*. Penis short, broad at the base, close to head-foot mass, sub-circular in section; penis ending truncated (Fig. 8E) or at mid of its length the penis may become narrower, terminating in a elongated extension (Fig. 8F); duct of penis broad, somewhat sinuous at base, more linear in its distal region.

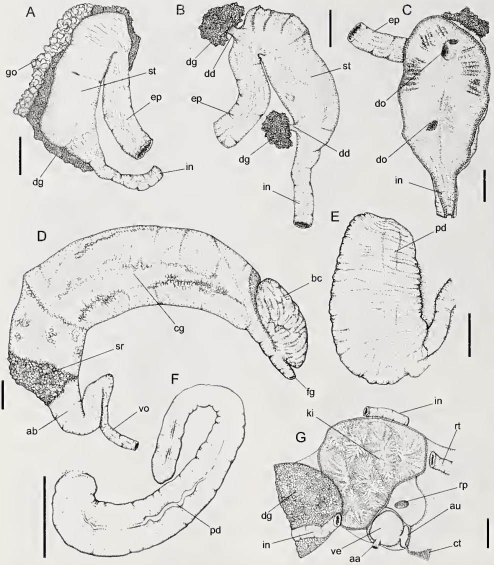

Figure 8. *Leucozonia ocellata.* **A**, Stomach in dorsal view; **B**, Stomach in ventral view; **C**, Internal view of stomach, esophagus partially opened longitudinally; **D**, Pallial oviduct in ventral view; **E–F**, Penis; **G**, Renopericardial region in ventral view opened longitudinally, part of posterior region of pallial cavity also shown. Abbreviations: **aa**, anterior aorta; **ab**, albumen gland; **au**, auricle; **bc**, bursa copulatrix; **cg**, capsule gland; **ct**, ctenidium; **dd**, duct of digestive gland; **dg**, digestive gland; **do**, digestive opening; **ep**, posterior esophagus; **fg**, female gonopore; **go**, gonad; **in**, intestine; **ki**, kidney; **pd**, penis duct; **rp**, renal aperture; **rt**, rectum; **sr**, seminal receptacle; **st**, stomach; **ve**, ventricle; **vo**, visceral oviduct. Scale bars: 1.0 mm.

Circulatory and excretory systems (Fig. 8G): As in *Leucozonia nassa*. Nephridial gland indistinct.

Leucozonia ponderosa Vermeij and Snyder, 1998 (Fig. 9)

Leucozonia ponderosa Vermeij and Snyder (1998: 117–119, figs. 1–4); Vermeij and Snyder (2002: 32, figs. 31–34, 41–42); Snyder (2003: 164, 317).

Leucozonia nassa auct non Gmelin, 1761: Leal (1991, in part).

Type locality:

Enseada dos Portugueses, Trindade Island, Brazil, 20°30'S, 29°20'W, depth 10m, rocky bottom.

Types:

Holotype: MORG 39298, Enseada dos Portugueses, Trindade Island, Brazil, 20°30'S, 29°20'W, depth 10m, rocky bottom, J. H. Leal, P. Bouchet col. 22/v/1987; paratype: MORG 39299, Enseada dos Portugueses, Trindade Island, Brazil, 20°30'S, 29°20'W, depth 10m, rocky bottom, J. H. Leal, P. Bouchet col. 22/v/1987.

Distribution:

Oceanic Islands of Trindade, off Espírito Santo state and Fernando de Noronha, off Pernambuco state, Brazil.

Examined material:

In addition to the types: Brazil: Fernando de Noronha Archipelago, off Pernambuco state: MNRJ 14607, Buraco do Inferno, depth 12–18m, P. M. S. Costa col. viii/2009 [1 specimen]; Trindade Island, off Espirito Santo state: MNRJ 5127, P. Andrada, S. Ypiranga, A. Coelho, J. Becker col. Xi/1957 [2 shells]; MNRJ 5128, Tartarugas Beach, J. Becker col. Xii/1965 [1 shell]; MNRJ 5129, Farfilhões Beach, J. Becker, A. B. Costa col. Xii/1965 [2 shells]; MNRJ 5132, Cabritos Beach, S. Ypiranga, J. Becker, A. Coelho col. x/1957 [3 shells]; MNRJ 5131, Cabritos Beach, J. Becker, col. xii/1958 [1 shell]; MNRJ 5136, Portugueses Beach, S. Ypiranga, A. Coelho, J. Becker col. x/1957 [2 shells]; MNRJ 5137, Cabritos Beach, B. Prazeres col. xii/1975–ii/1976 [3 shells]; MNRJ 5138, Galheta Beach, B. Prazeres col. xii/1975–ii/1976 [6 shells]; MNRJ 5139, Príncipe Beach, B. Prazeres col. xii/1975 [6 shells]; MNRJ 5220, Cachoeira Beach, J. Becker col. i/1959 [2 specimens]; MNRJ 5134, Andrada Beach, J. Alberto col. i/1959 [2 shells]; MNRJ 5130, Tartarugas Beach, J. Becker col. xii/1958 [4 shells]; MNRJ 5135, Andrada Beach, J. Becker col. xii/1958–i/1956 [3 shells]; MNRJ 5133, B. Lobo expedition, P. P. Velho, J. Domingues col. 1916 [8 shells].

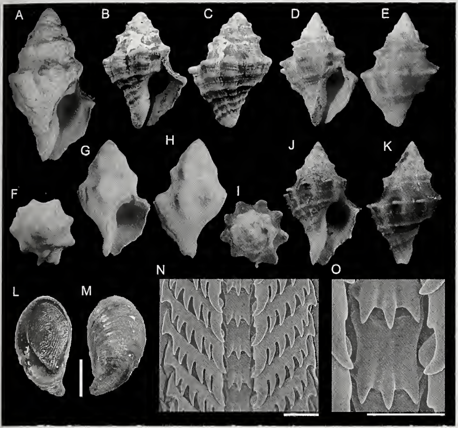

Figure 9. *Leucozonia ponderosa.* **A**, 47.3 mm (holotype, MORG 39599); **B–C**, 37.3 mm (MNRJ 5137); **D–E**, 31.4 mm (MNRJ 5138); **F–H**, 44.2 mm (MNRJ 5220); **I–K**, 28.2 mm (MNRJ 14607). **L**, Operculum in inner view; **M**, Operculum in outer view; **N**, Radula in general view of median rows; **O**, Detail of rachidian teeth of radula. Scale bars: 73–74: 5.0 mm; 75–76: 50 µm.

Description:

Shell (Figs. 9A–K): Shell somewhat elliptical fusiform, maximum length of 46.6 mm, ~ twice as long as wide; color chestnut to brown, its nodes slightly faded. Spire high with angle varying from 75° to 80°, spire length about half of total shell length. Protoconch small, with two whorls, smooth; terminal varix low. Teleoconch with up to five and a half whorls, shaped convex by two angulations, one at shoulder and the other at central cord, sub-sutural ramp concave, suture impressed; base of shell concave. Spiral sculpture of five to seven main cords: one at shoulder, one marking a central cord and one between these two, another two to four cords at base; several other fine cords throughout the entire teleoconch. Axial sculpture of 8 to 10 strong ribs forming elevated nodes where they cross the three upper spiral cords, shoulder nodes more prominent. Aperture roughly elliptical to slightly pyriform, height ~ twice the width. Columella bearing three to four small folds in its center. Outer lip thin, marked internally by nine to 12 beaded lirae, small and blunt labral tooth at end of central cord may be present. Siphonal canal small, its length ~1/3 of total aperture height. Siphonal fasciole distinct. Umbilical slit absent.

Soft Parts: as in *Leucozonia nassa*. Male genital system not examined due to lack of male specimens.

Operculum (Figs. 9L–M): as in *Leucozonia nassa*.

Radula (Figs. 9N–O): radula long and thin; radular sac extending beyond the posterior end of odontophore; rachidian tooth straight, rectangular, its base with a concave outline and its cusped margin of convex outline, bearing three sharp cusps, the central one slightly larger; lateral tooth wider then long, bearing six centrally recurved cusps, the innermost being about twice as long as the others which have approximately the same size, except for the outermost, slightly smaller; lateral margin acute, ending in a more external, last cusp; occurrence of a denticle-like projection on inner margin of the innermost cusp.

DISCUSSION

Vermeij and Snyder (2002) revised the taxonomy of *Leucozonia nassa*, examining shells from several locations from North Carolina to Brazil, and concluded that it is a widely variable species, as evidenced by its many synonyms. According to Vermeij and Snyder (2002), the shell of *L. nassa* is distinguished by a combination of characters not found in any other species of *Leucozonia*, and three distinct geographical forms can be recognized. These forms had been identified as three different species by Vermeij (1997), and three subspecies by Abbott (1958): the typical *L. nassa nassa* (Figs. 1L–M), which occurs in Caribbean islands and from North Carolina to Florida and the Gulf of Mexico; *L. nassa leucozonalis* (Figs. 1E–K), found in offshore Brazilian waters, off Bahia and the islands of Fernando de Noronha, Atol das Rocas, and Trindade; and *L. nassa brasiliana* (Figs. 1A–D), from the southeastern to northeastern Brazilian coast. Vermeij and Snyder (2002) considered *L. leucozonalis* as a distinct species, not occurring in Brazilian waters, which closely resembles the form of *L. nassa leucozonalis* (*sensu* Lamarck, 1822) from oceanic islands of Brazil.

Another oceanic species, *Leucozonia ponderosa* (Fig. 9), was described as distinct from *L. nassa* on the basis of shell characters: more-prominent spiral sculpture and a somewhat heavier nodulated shell (Vermeij and Snyder 1998). Vermeij and Snyder (2002) reinforced their original conclusion that *L. ponderosa* is consistently different, but closer to *L. nassa*. According to these authors, in the typical form of *L. nassa* (Figs. 1L–M), the two upper main cords lie very close together, whereas in *L. ponderosa* the second cord lies approximately midway between the shoulder cord and the central cord (Fig. 9K).

The conclusions by Vermeij and Snyder (2002) were based on only five shells of *Leucozonia ponderosa*, all from Trindade Island. Additional material of *L. ponderosa* (Figs. 9B–K) examined here, including a specimen from Fernando de Noronha (Figs. 9I–K), exhibited the differences pointed out by Vermeij and Snyder (2002). Several lots in the MNRJ Mollusk Collection, collected at Trindade Island in the 1950–1970s and determined by Leal (1991) as *L. nassa*, were re-examined for this study and determined as *L. ponderosa*. Rios (2009) considered *L. ponderosa* as a synonym of *L. nassa* without further argumentation.

Mallard and Robin (2005: pl. 56) illustrated a shell from Brazil, as *Leucozonia ponderosa*, which is quite distinct, with very pronounced nodes and a markedly tapered spire. The identification of this shell is dubious. Mallard and Robin (2005: 20) stated that *L. ponderosa* ranges from the West Indies to Brazil, without further comments.

The anatomy of *Leucozonia nassa* from the coast of Brazil (*L. nassa brasiliana sensu* d'Orbigny, 1841) (Figs. 1A–D) was studied by Marcus and Marcus (1962), based on specimens from Ubatuba, São Paulo state. They accomplished a fairly detailed morphological description of this species, including histological sections. In general, the anatomical results obtained here from specimens from the states of Rio de Janeiro (Figs. 1A–B) and Espírito Santo (Figs. 1C–D) agrees with those obtained by Marcus and Marcus (1962). Examination of specimens from Trindade, corresponding to the form from the oceanic islands of Brazil determined by Vermeij and Snyder (2002) as *L. leucozonalis* (*sensu* Lamarck, 1822) (Figs. 1I–K), also showed no anatomical differences from the specimens from Rio de Janeiro and Espírito Santo states.

Two female specimens of *Leucozonia ponderosa* were studied, and their anatomy is identical to that of *L. nassa*. The only minor differences are in the radula (Figs. 9N–O), which has a well-marked denticle on the inner margin of the lateral teeth (Fig. 9O), similar to *L. ocellata* (Fig. 5G), whereas in *L. nassa* it is barely distinguishable (Fig. 1Q). The presence of this denticle was reported by Bandel (1984) in *L. ocellata*, but not in *L. nassa*.

In spite of the lack of anatomical differences, and considering that no male specimens of *Leucozonia ponderosa* were available to evaluate possible differences in the male genital system, we continue to recognize two distinct species, based on the shell differences described by Vermeij and Snyder (2002).

In contrast, *Leucozonia ocellata* shows marked anatomical differences from *L. nassa* and *L. ponderosa*. The rhynchostome of *L. ocellata* is emarginated by lip-like folds (Fig. 7A: ry), whereas in *L. nassa* the rhynchostome is slit-like (Fig. 3A: ry); the siphon has three distinct folds (Fig. 6F) in *L. ocellata*, whereas in *L. nassa* it is smooth (Figs. 2C–D: si); the osphradium of *L. ocellata* is proportionately larger (Fig. 6C: os), with its filaments triangular and bearing acute projections, in which the left ones are smaller (Fig. 6E: of), whereas in *L. nassa* the osphradium filaments are somewhat curved and about the same size (Fig. 2E: of); the anterior esophagus is slightly curved in *L. ocellata* (Fig. 7B) and straight in *L. nassa* (Fig. 3B); the seminal receptacle and the bursa are proportionately larger in *L. ocellata* (Fig. 8D: bc, sr) than in *L. nassa* (Fig. 4D: bc, sr); the cement gland of *L. nassa* is a single sac with nearly smooth walls (Fig. 2B: fc), as described by Marcus and Marcus (1962), rather different from that of *L. ocellata*, which has a posterior folded branch (Fig. 6B: fc).

All specimens of *Leucozonia nassa* examined have an elongated penis bearing a long thread-like terminal extension (Fig. 4E); in *L. ocellata* the penis may be truncated, as in specimens from Abrolhos in the state of Bahia (Fig. 8E), or elongated, as in those from Rio de Janeiro (Fig. 8F).

The shell of *Leucozonia ocellata* differs from that of *L. nassa* by its usually smaller size, less-numerous beaded lirae on the inner side of the outer lip, and distinct highlighted whitish nodes. This species has no labral tooth, and specimens incrusted with coralline algae may be difficult to distinguish from small individuals of *L. nassa* (Leal 1991), which is known for its variable shell sculpture and distinct geographical patterns, as pointed out by Vermeij and Snyder (2002). Although *L. ocellata* shows some variation in shell form and sculpture, Vermeij and Snyder (2002) were unable to identify geographical patterns in this species.

Anatomy of Fasciolariidae

Kosyan *et al.* (2009) studied the anatomy of fasciolariids based on eight species in seven genera from three subfamilies: *Turrilatirus turritus* (Gmelin, 1791), *Pustulatirus mediamericanus* (Hertlein and Strong, 1951), *Latirus polygonus* (Gmelin, 1791), *Peristernia nassatula* (Lamarck, 1822), *Peristernia ustulata* (Reeve, 1847), *Opeastoma pseudodon* (Burrow, 1815), *Fusinus tenerifensis* Hadorn and Rolán, 1999, and *Fasciolaria lignaria* (Linnaeus, 1758). Although no species of *Leucozonia* was studied, and the work by Marcus and Marcus (1962) on *L. nassa* was not mentioned, these authors contributed a thorough anatomical description for the family as a whole.

The following characters were observed by Kosyan *et al.* (2009) for Fasciolariidae, and are in agreement with the *Leucozonia* species studied here:

The asymmetrical osphradium, more prominent in *Leucozonia ocellata* (Fig. 6E: of), and the stomach without a posterior mixing area (Figs. 4C, 8C).

The straight proboscis, never coiled within the proboscis sheath, as described by Kosyan *et al.* (2009) for fasciolariids, was also found in *Leucozonia* (Figs. 3B, 7B: pb). The relationship of the proboscis length to the buccal mass varied in the species studied by Kosyan *et al.* (2009), being short (equal to the buccal mass) in most of the species, as in the species of *Leucozonia* studied here.

Kosyan *et al.* (2009) described the salivary glands of fasciolariids as either separate masses or fused beneath the nerve ring. In *Leucozonia*, the two branches of the salivary glands, although adjacent, are not fused (Figs. 3B, 7B: sg) as also reported by Marcus and Marcus (1962) for *L. nassa*. In accordance to the fasciolariid species described by Fraussen *et al.* (2007), the ducts of the salivary glands in *Leucozonia* are embedded in the esophagus wall (Figs. 3C, 7E: sd), although Kosyan *et al.* (2009) reported the ducts passing freely along the esophagus except in *Latirus polygonus*.

According to Kosyan *et al.* (2009), the only morphological character differentiating fasciolariids from buccinids is the proboscis retractor muscles, which consist of one single or paired tufts of muscle fibers in fasciolariids and multiple tufts of muscle fiber in the buccinids studied by Kosyan *et al.* (2009) and Kosyan and Kantor (2009). In the three species of *Leucozonia* studied here, there was a single broad muscle (Figs. 3B and 7B: rm).

The *Leucozonia* species studied here possess the diagnostic anatomic features according to Fraussen *et al.* (2007): the broad multicuspid lateral teeth and a small and narrow rachidian tooth, paired or single powerful proboscis retractor muscle, salivary ducts embedded in the lateral folds of the esophagus wall and the stomach without a posterior mixing area (caecum), low folds on the inner wall and transverse striations on the longitudinal fold.

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Revision of the genus *Pazinotus* (Gastropoda, Muricidae) from Brazil

Paulo Márcio Santos Costa and Alexandre Dias Pimenta

Departamento de Invertebrados, Museu Nacional, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista, São Cristóvão, CEP 20940-040, Rio de Janeiro, Rio de Janeiro, Brazil

Correspondence, Alexandre Dias Pimenta: adpimenta@yahoo.com.br

Abstract: A revision of the genus *Pazinotus* from Brazil revealed the occurrence of three species: *Pazinotus stimpsonii* (Dall, 1889a), with a wide geographical range in the western Atlantic, including Florida, the Caribbean, and the southeast coast of Brazil; *Pazinotus bodarti* (Costa, 1993), restricted to the southeast coast of Brazil; and *Pazinotus gili* new species, restricted to the Canopus Bank on the northeast coast of Brazil. *Pazinotus gili* new species is distinguished by its smaller size, less-pronounced teeth on the outer lip, and the absence of spiral sculpture on the teleoconch whorls. A microscopic sculpture is described for species of *Pazinotus*, consisting of microgranules on the protoconch and series of spiral pitted lines on the teleoconch surface or in the intritacalx of *Pazinotus gili* new species.

Key words: taxonomy, western Atlantic, Muricopsinae, Muricoidea

The genus *Pazinotus* (Vokes, 1970) comprises about 12 recent and fossil muricid species distributed in the Indo-Pacific, New Caledonia, and in the western Atlantic, where four species are listed (Houart 2006): *Pazinotus stimpsonii* (Dall, 1889a), *P. bowdenensis* (Vokes, 1970), *P. bodarti* (Costa, 1993), and *P. goesi* (Houart, 2006). Two of these are recorded from the southeast coast of Brazil: *P. bodarti*, described from shallow water, and *P. stimpsonii*, recorded live from depths of 52–105 m (Houart 1991a; Leal 1991) and dead from 1500–1575 m (Houart, 1991a).

Recent collecting off the northeast coast of Brazil resulted in the discovery of an undescribed species of *Pazinotus*, which led us to undertake a taxonomic revision of the species of *Pazinotus* occurring in Brazil.

MATERIAL AND METHODS

The new species described herein is based on a sample collected on the Canopus Bank, state of Ceará, in 2005, among biogenic substrate, between 240–260 m depth. Material of *Pazinotus bodarti* was obtained from collectors in the state of Espírito Santo.

The identification of the species was based on the original and subsequent descriptions as well as on examination of the type material. During examination of paralectotypes of *Pazinotus stimpsonii*, it was noted that the shells still contained dry fragments of soft parts of the animal. An attempt was made to rehydrate the soft parts in order to extract the radulae, but only the operculum was obtained. For the other two species, radulae were recovered by dissolving head tissues in potassium hydroxide (KOH) for different periods; the radulae were cleaned by ultrasonic transmission and mounted for photography in a Jeol JSM-6390LV scanning electron microscope (SEM). Radular terminology was adapted from Kool (1993) and Herbert *et al.* (2007). Some aspects of the head-foot mass and penis of the new species were described after examination using a stereomicroscope with the aid of a drawing tube. The terminology used for the description of the spiral sculpture of the teleoconch whorls and outer lip denticles was based on Merle (2001, 2005) and Houart (2006): P1: shoulder cord; P2–P4: primary cords; s1–s4: secondary cords; ADP: adapical siphonal primary cord; MP: median siphonal primary cord; ABP: abapical siphonal primary cord; ID: infrasutural denticle; D1–D5: abapical denticles on outer lip.

Abbreviations used throughout the text: CPMSC: Private collection of Paulo Márcio Santos Costa, Rio de Janeiro, Brazil; FURG: Fundação Universidade do Rio Grande, Rio Grande do Sul, Brazil; HABITATS: Habitats Project-Campos Basin Environmental Heterogeneity by CENPES/PETROBRAS; IBUFRJ: Instituto de Biologia / Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; MD55: Expedition Marion Dufresne, 1987; MNHN: Muséum national d'Histoire naturelle, Paris, France; MNRJ: Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; MORG: Museu Oceanográfico Prof. Eliézer de Carvalho Rios, FURG, Rio Grande, Rio Grande do Sul, Brazil; MZSP: Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil; REVIZEE: Recursos Vivos da Zona Econômica Exclusiva; SMNH: Swedish Museum of Natural History, Stockholm, Sweden; USNM: National Museum of Natural History, Washington, District of Columbia, U.S.A.

SYSTEMATICS

Family Muricidae Rafinesque, 1815
Subfamily Muricopsinae Radwin and D'Attilio, 1971
Genus *Pazinotus* Vokes, 1970

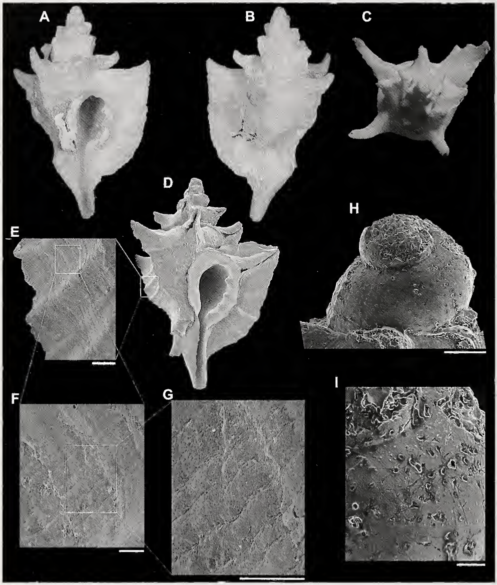

Figure 1. *Pazinotus stimpsonii*. **A–B**, lectotype USNM 87087; **C–I**, paralectotypes USNM 887350. **A–D**, whole shells (lengths: **A–B**, 12.0 mm; **D**, 9.7 mm); **C**, apical view; **E–G**, detail of teleoconch microsculpture; **H**, protoconch; **I**, detail of protoconch sculpture. Scale bars: **E**, **H**, 200 µm; **F–G**, **I**, 50 µm.

Poirieria (*Pazinotus*) Vokes (1970: 27). Type species by original designation: *Eupleura stimpsonii* Dall, 1889a. Barbados.

Diagnosis

Shell biconic-fusiform, spire high, about half shell length. Aperture ovate, four or five prominent denticles on outer lip and two or three weak plica-like pustules on abapical end of columella, Siphonal canal narrow, short. Teleoconch sculpture consists of axial winglike varices, forming weakly to moderately developed spines recurving backwards, and very weak to moderate spiral cords; microscopic sculpture formed by series of pitted lines. Intritacalx may be present. Protoconch globose of about 1.5 whorls with microgranules more densely concentrate on its anterior half.

Pazinotus stimpsonii (Dall, 1889a) (Figs. 1–2)

Eupleura stimpsonii Dall (1889a: 204)

Eupleura stimpsonii: Dall (1889b: 120, pl. 42, fig. 3; 1890: 331, pl. XI, fig. 3); Smith (1939: 17, pl. 13, fig. 24); Abbott (1954: 209, fig. 47a); Abbott (1968: 126, fig. 9)

Poirieria (*Pazinotus*) *stimpsonii*: Vokes (1970: 28, pl. 5, figs. 6a, 6b (lectotype designation), 7a, 7b (paralectotype MCZ); Abbott (1974: 186, fig. 1947); Houart (1991a: 31, fig. 32); Vokes (1992: 37, pl. 7, fig. 9)

Pazinotus stimpsonii: Radwin and D'Attilio (1976: 87); Fair (1976: 79, pl. 15, fig. 202); Leal (1991: 138); Rios (1994: 110, pl. 36, fig. 461); Houart (2006: figs. 6–9, 11); Houart and Héros (2008: 472–473, figs. 9D, 10A)

Type material

Lectotype designated by Vokes (1970: 28): USNM 87087: length: 12.0 mm, width: 7.0 mm, 5 teleoconch whorls (measurements according to Dall, 1889a); four paralectotypes: USNM 887350: paralectotype 1: length: 10.7 mm, width: 8.8 mm, 4.6 teleoconch whorls; paralectotype 2: length: 7.5 mm, width: 5.7 mm, 4.0 teleoconch whorls; paralectotype 3: length: 9.8 mm, width: 6.6 mm, 4.5 teleoconch whorls; paralectotype 4: length: 9.7 mm, width: 5.9 mm, 4.5 teleoconch whorls; four lectotypes: MCZ 7310 (studied by Vokes, 1970). All paralectotypes from type locality.

Type Locality

Off Barbados, 100 fathoms (183m), Blake Expedition (station unnumbered), about 13°08′N, 59°30′W.

Material examined

The lectotype photograph and USNM 887350, four paralectotypes; MNRJ 16291, off Rio de Janeiro state, HABITATS station I-04 (21°9′5.7″S, 40°16′13.4″W, 101 m), 07/iii/2009 [1 shell].

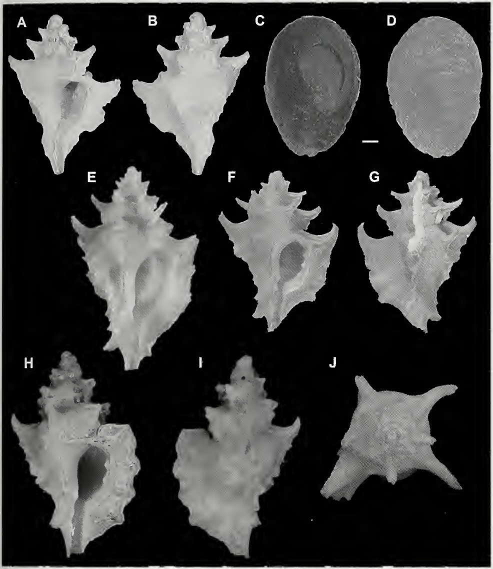

Figure 2. *Pazinotus stimpsonii.* **A–D**, paralectotypes USNM 887350; **E**, shell from east Panama (photo and collection R. Houart); **F–J**, shells from southeast Brazil (**F–G**, MNHN; **H–J**, MNRJ 16291). **A–B**, **E–I**, whole shells (lengths: **A–B**, 7.5 mm; **E**, 10.6 mm; **F–G**, 7.0 mm; **H–I**, 7.3 mm); **J**, apical view; **C**, operculum in inner view; **D**, operculum in outer view. Scale bar: 200 μm.

Distribution

Florida to Barbados (Radwin and D'Attilio 1976), Virgin Islands (18°65′W) (Houart 2006); east Panama (R. Houart, pers. comm.); southeast coast of Brazil: off Espírito Santo state (around 19°S–21°S, 37°50′W–38°W), (Houart 1991a; Leal 1991); off Rio de Janeiro state (around 21°9′S, 40°16′W). Living in 52 to ~ 200 m (Dall 1889a; Houart 1991a).

Redescription

Shell small (lectotype 12.0 mm in length *fide* Dall, 1889a: 204), biconical, spinose, spire high, about ⅓ of total shell length, milky-white. Protoconch paucispiral, with about 1.5 whorls, white, globose, with strong terminal lamellae, slightly erect; apparently smooth, with well-spaced sparse microgranules. Teleoconch of five whorls (lectotype), shouldered, with spinose varices. Suture impressed (subsutural shelf straight). Axial sculpture formed by fine growth lines and strong lamellate varices (four on last whorl of holotype), each varix forming a moderately long grooved spine recurved backwards; area between adjacent axial varices slightly convex in apical view; last varix forming a winglike spinose lamella on outer lip, ornamented with four radial ribs corresponding to spiral sculpture. Spiral sculpture formed by four or five primary cords (P1–P5), more conspicuous on lamellar portion of the varices; secondary cords (s1–s4) very tiny, hardly discernable. Shell surface opaque, ornamented by spiral series of microscopic pitted lines, formed by very small and close holes, spacing between each pitted line of irregular width, forming slightly convex areas and covered by small holes, more widely spaced than in pitted lines; on concave areas between spiral cords, the pitted lines are closer to each other. Aperture ovoid, rounded adapically, interior glossy white, columellar lip well reflected into parietal region; siphonal canal moderately long (about 1/3 of total shell length), open, straight, with same length as aperture. Dorsally recurved; end of former canal projecting from it at the left. Outer lip sharp, inner face bearing usually five strong denticles within (ID, D1, D3–D5); D2 absent or hardly discernible; inner lip prominent, adherent posteriorly, with two or three small nodes on abapical region. Anal notch indistinct.

Operculum corneous, ovate, covering entire shell aperture; external surface with a terminal nucleus and concentric growth lines; inner surface attachment area covering less than 50% of total area of operculum, with a single horseshoe-shaped scar, located on right side of posterior area.

Radula of rachiglossate type; rachidian tooth of three-dimensional form, rectangular, width about two times its height, with five pointed cusps of nearly equal size, central cusp wider, lateral cusps slightly thinner and shorter, marginal area broad, with wide and acute marginal cusp, marginal edge of tooth somewhat concave, base somewhat sinuous; lateral

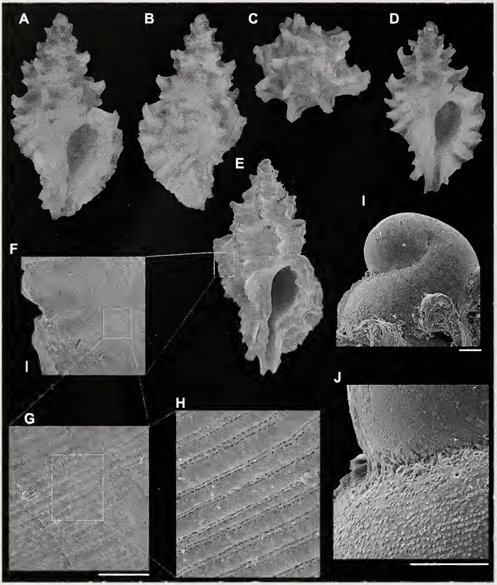

Figure 3. *Pazinotus bodarti*. **A–C**, holotype MORG 32000; **D**, paratype MORG 32001; **E–J**, MNRJ 11905. **A–E**, whole shells (lengths: **A–B**, 12.2 mm; **D**, 9.0 mm; **E**, 10.2 mm); **F–H**, detail of teleoconch microsculpture; **I**, protoconch; **J**, detail of protoconch sculpture. Scale bars: 100 μm.

teeth sickle-shape, broader at the base. Description based on illustration by Houart and Héros (2008: 472, fig. 9D), of specimen from Virgin Islands (SMNH 71734).

Pazinotus bodarti (Costa, 1993)
(Figs. 3–4)

Poirieria (*Pazinotus*) *bodarti* Costa (1993: 50, figs. 1–3); Houart (1994: 35).

Redescription

Shell small (holotype 12.2 mm in length), biconic, not polished, spire high, about ½ of total shell length, light-brown with dark-brown stains between the spines. Protoconch paucispiral, with about 1.5 whorls, white, globose, with a well-marked terminal lamellae, first whorl partially smooth, with irregular blotches of microgranules closer to suture, very few and sparse above it, becoming numerous and dense, covering entire surface of last half of last protoconch whorl, arranged in approx. spiral rows. Teleoconch of five whorls (holotype), shouldered, with spinose varices. Suture impressed (subsutural shelf very short and slightly concave). Axial sculpture formed by fine growth lines and strong lamellate varices, seven on last whorl of holotype; areas between adjacent axial varices concave in apical view; last varix forming winglike spinose lamella on outer lip. Spiral sculpture on last whorl formed by four primary cords (P1–P4) forming short recurved canaliculated spines, on each varix; secondary cords absent in most specimens examined, but present in at least one shell as very tiny cords; siphonal canal with visible ADP, MP and ABP in some specimens, but hardly distinguishable in most shells. Shell surface opaque, ornamented with spiral series of microscopic double pitted lines, formed by very small and closely set holes, space between each double pitted line forming slightly convex smooth area about four times the width of the double line; in the concave areas between spiral cords, the double lines are closer. Aperture ovoid, rounded adapically, interior glossy white, columellar lip reflected into parietal region; siphonal canal short (about 1/5 of total shell length), open, straight, slightly curved dorsally, the end of the former canal projecting from it at the left. Outer lip sharp, inner face bearing six strong denticles within (ID, D1–D5); inner lip prominent, adherent adapically, with a small callus abapically. Anal notch indistinct.

Operculum horny, ovate-elongated, covering entire shell aperture; external surface with a terminal nucleus and concentric growth lines; inner surface attachment area covering less than 50% of total area of operculum.

Radula of rachiglossate type; rachidian tooth of three-dimensional form, rectangular, width about three times its height, with five pointed cusps of nearly equal size, central cusp slightly wider, lateral cusps slightly thinner and

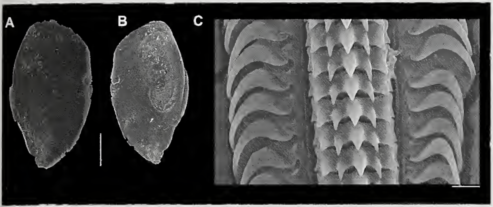

Figure 4. *Pazinotus bodarti.* **A**, operculum in outer view; **B**, operculum in inner view; **C**, radulae in dorsal view. Scale bars: **A–B**, 500 µm; **C**, 10 µm.

longer, marginal area broad, with wide and acute marginal cusp, marginal edge of tooth somewhat concave, base somewhat sinuous; lateral teeth sickle-shaped, broader at the base.

Type material

Holotype: MORG 32000 (length: 12.2 mm, width: 6.3 mm, 5 whorls); paratypes: MORG 32001 (length: 9.0 mm, width: 5.3 mm, 4.5 whorls); MNRJ 21650, ex-CPMSC 1650 (length: 10.2 mm, width: 5.6 mm, 5 whorls); MZSP 27959 (length: 7.15 mm, width: 4.0 mm, 4 whorls).

Type Locality

Between Escalvada Island and Rasa Island (20°41′S, 40°22′, 25 m depth), off Guarapari, Espírito Santo State, Brazil.

Material examined

In addition to the types: Espírito Santo state: MNRJ 12907, off Linhares (19°26′16″S, 39°15′22″, 61.8 m depth), x/2003 [1 shell]; MNRJ 12908, off Linhares (19°26′25″S, 39°20′03″W, 46 m depth), x/2003 [1 shell]; MNRJ 11906, off Vitória (30 m depth), xi/2003, collected by fishing boat [2 specimens]; MNRJ 11905, off Guarapari (30 m depth), iv/2006, fishermen coll. [3 specimens]; Rio de Janeiro state: MNRJ 12066, off Cabo Frio (23°04′S, 40°59′W), Navio Astro Garoupa coll. [3 shells], 17/xii/2004; IBUFRJ 13087, REVIZEE station vv38 (19°44′S, 38°22′W, 71.4 m), Navio Astro Garoupa coll., 29/ii/1996 [3 shells]; MNRJ 16292, HABITATS station H-04 (21°42′49″S, 40°10′21″, 98 m) [1 shell]; MNRJ 16293, HABITATS station H-03 (21°43′5″S, 40°11′36″W, 73 m), 09/iii/2009 [1 shell].

Distribution

Southeast off Brazil, Espírito Santo and Rio de Janeiro states. Up to 98 m depth.

Remarks

After examining the types of *Pazinotus bodarti*, we noticed some confusion in the illustrations and legends of the original description (Costa 1993). The figured shells of *P. bodarti* in Costa (1993: 49, figs. 1, 2) cited as holotype and paratype 2, respectively, actually correspond to the same shell, the paratype 2 (CPMSC 1650); this lot is now being transferred to the Mollusca Collection of Museu Nacional, under the number MNRJ 21650. The holotype of *P. bodarti* (MORG 32000) is a larger shell, herein illustrated for the first time (Figs. 3A–C).

Pazinotus gili new species
(Figs. 5–6)

Type material (all from type locality)

Holotype: MNRJ 11037 (length: 6.35 mm, width: 3.55 mm, 3.5 whorls); five paratypes: MNRJ 11063 (paratype 1: length: 6.0 mm, width: 3.65 mm, 3.6 whorls; paratype 2: length: 6.35 mm, width: 3.55 mm, 3.5 whorls; paratype 3: length: 5.2 mm, width: 3.05 mm, 3.2 whorls; paratype 4: length: 3.75 mm, width: 2.7 mm, 2.4 whorls; paratype 5: length: 3.7 mm, width: 2.3 mm, 2.5 whorls); three paratypes: MZSP 70335 (paratype 6: length: 5.55 mm, width: 3.35, 3.5 whorls; paratype 7: length: 6.15 mm, width: 3.35 mm, 3.5 whorls; paratype 8: length: 5.5 mm length, width: 3.35 mm, 3.25 whorls); two paratypes: MZSP 70282 (paratype 9: length: 6.05 mm, width: 3.75 mm, 3.5 whorls; paratype 10: length: 6.2 mm, width: 3.8 mm, 3.5 whorls); MNRJ 11008 (paratype 11: broken shell and dissected specimen).

Type Locality

South slope of the Canopus Bank, off Ceará state (02°14′25″S, 038°22′ 50″W); 240–260 m depth.

Distribution

Known only from the type locality.

Diagnosis

Shell small for the genus; weakly-sculptured, without spiral cords; both spines and outer lip denticulation very weak.

Description

Shell small (holotype 6.35 mm in length), biconic, spire high, about ½ of total shell length, grayish-white. Protoconch

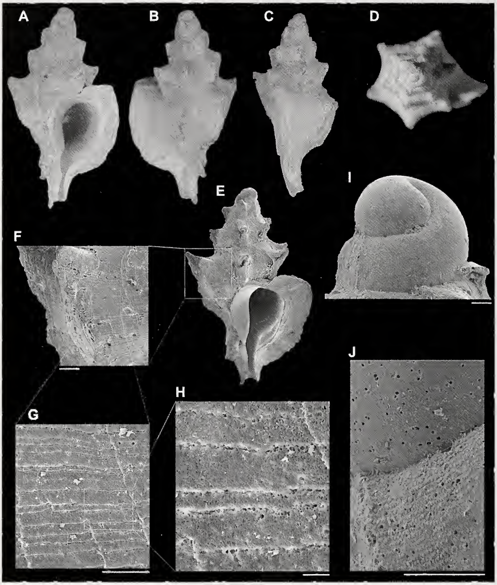

Figure 5. *Pazinotus gili.* **A–D**, holotype MNRJ 11037; **E–H**, paratype MZSP 37194; **I–J**, paratype MNRJ 11063; **A–E**, whole shells (lengths: **A–C**, 6.35 mm; **E**, 6.35 mm); **F–H**, detail of teleoconch microsculpture; **I**, protoconch; **J**, detail of protoconch sculpture. Scale bars: **F**, 200 μm; **G–H**, 100 μm; **I**, 10 μm.

white, globose, paucispiral, with about 1.5 whorls, with a well-marked terminal straight lamellae; first whorl partially smooth, with irregular blotches of microgranules below the suture, but very few and sparce above it, becoming numerous and dense, covering entire surface of last half of last whorl, where they are arranged in approx. spiral rows. Teleoconch of 3.5 whorls (holotype), shouldered, with spinose varices. Suture impressed (subsutural shelf somewhat concave). Axial sculpture formed by fine growth lines and strong varices; five varices on last whorl of holotype, each varix forming a short grooved spine slightly recurved backwards; area between adjacent axial varices slightly concave in apical view; last varix forming winglike spinose lamella on outer lip, with radial ribs. Spiral sculpture with two very tiny primary cord (P1–P2), hardly discernible, forming a short grooved spine slightly recurved. Shell surface opaque, covered by grayish-white intritacalx, ornamented with spiral series of microscopic pitted lines, formed by very small and closely set holes, space between each pitted line forming slightly convex areas of variable width and covered by more widely spaced holes than in the pitted line. Aperture ovoid, rounded adapically, interior glossy white, continuously marginate except at the open narrow canal; columellar lip well reflected into parietal region; siphonal canal short (about 1/5 of total shell length) slightly curved dorsally, the end of the former canal projecting from it at the left. Outer lip sharp, inner face bearing three small weak denticles within (D3–D5); inner lip prominent, attached posteriorly in the columella, with a small callus in the anterior part of the columella as resulting from the deposition of earlier siphonal canals. Anal canal indistinct.

Head-foot (male): head poorly-differentiated, eyes located laterally and at middle of somewhat short cephalic tentacles. Penis almost of same length of entire head-foot mass, broad, with a terminal recurved papilla and a grooved aperture; penis duct somewhat sinuous.

Operculum corneous, ovate, covering entire shell aperture; external surface with a terminal nucleus and concentric growth lines; inner surface attachment area covering less than 50% of total area of operculum, not positioned centrally.

Radula of rachiglossate type; rachidian tooth of three-dimensional form, trapezoidal, width about two times its height, with five pointed cusps of nearly equal size, central cusp slightly wider, lateral cusps thinner, marginal area broad, with wide and acute marginal cusp, marginal edge of tooth somewhat concave, base somewhat sinuous; lateral teeth sickle-shaped, broader at the base.

Etymology

This species is named in honor of Mr. Antonio Gil Bezerra owner of INACE Shipyard (Industria Naval do Ceará).

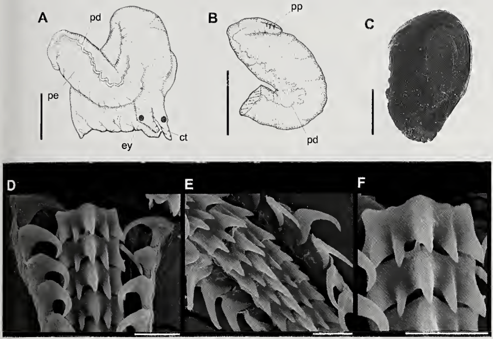

Figure 6. *Pazinotus gili.* Paratype MNRJ 11008: **A**, head-foot in dorsal view, male; **B**, penis; **C**, operculum in outer view; **D–E**, radulae in dorsal view; **F**, detail of rachidian teeth. Scale bars: **C**, 500 µm; **D–F**, 10 µm. Abbreviations: **ct**, cephalic tentacle; **ey**, eye; **pd**, penis duct; **pe**, penis; **pp**, penis papilla.

Remarks

Pazinotus gili new species (Figs. 5–6) is very similar to *P. stimpsonii*, but, except for the series of microscopic pitted lines (Figs. 5F–H), has a smooth shell surface between the varices (Figs. 5A–C, E), and only one or two spiral cords much tinier than those in *P. stimpsonii* (Figs. 1A, D–E; 2A–B, E–I), *P. bodarti* (Figs. 3A–B, D–E), and *P. goesi*; also, the denticulations on the outer lip of the aperture are very weak (Figs. 5A, E) compared to those of *P. stimpsonii* (Figs. 1A, D; 2E–F, H) and *P. bodarti* (Figs. 3A, D–E), though they are stronger than in *P. goesi*. The spines of *P. gili* are shorter and less curved (Figs. 5A–C, E) than in *P. stimpsonii* and *P. goesi*, especially the apertural spines.

We did not utilize eroded shells in *Pazinotus gili*, since all specimens were collected alive, allowing us to observe the well-preserved intritacalx (Figs. 5A, E–F).

At first glance, one might consider *Pazinotus gili* as a juvenile of *P. stimpsonii*. However, juvenile specimens of *P. stimpsonii* with the same number of whorls and approx. the same length as the holotype of *P. gili* already have the well-marked spiral cords (Figs. 2A–B, F–G), elongate spines and in some shells well-developed denticles (Figs. 2F–G).

Pazinotus gili can be distinguished from the other species described from Brazil, *P. bodarti* (Costa, 1993), by its less-sculptured shell and the light-brown color of the shell of *P. bodarti* (Figs. 3A–E).

DISCUSSION

Prior to this study, two species of *Pazinotus* were reported from Brazil. The records of *P. stimpsonii* by Houart (1991a) and Leal (1991) were based on material collected during the RV 'Marion Dufresne' MD55 oceanographic expedition along the Vitória-Trindade Chain (a series of oceanic islands and seamounts off southeastern Brazil). In a paper that described the muricids from that expedition, Houart (1991a) listed four juvenile shells of *P. stimpsonii* collected alive from 85–105 m and dead from depths down to 1,575 m. Only one shell of these was illustrated (Houart 1991a: 30, fig. 32), measuring 6.2 mm in length. Leal (1991), based on the muricid material from the same expedition, listed two shells collected in 52 m and one in 85–105 m. All muricid material from the MD55 expedition is deposited in the MNHN collection (J. H. Leal, pers. comm.). One of them is illustrated herein (Figs. 2F–G) and is very similar to the shell illustrated by Houart (1991a: fig. 32).

A new record of *Pazinotus stimpsonii* in Brazil is established herein, from off Macaé in the state of Rio de Janeiro, based on a shell collected dead, from around 100 m depth (Figs. 2H–J).

The systematic classification of *Pazinotus* has been somewhat controversial at both the generic and subfamilial levels. *Pazinotus* was proposed as a subgenus of *Poirieria* by Vokes (1970) to include *Eupleura stimpsonii* Dall, 1889a (the type species of *Pazinotus*), as well as a fossil species from Jamaica, *Poirieria* (*Pazinotus*) *bowdenensis* Vokes, 1970. Subsequently, several other fossil and living species have been described or transferred to *Pazinotus* (Poorman 1980; Houart 1985, 1991b, 2006; Vokes 1992; Costa 1993). These attributions gave *Pazinotus* a wide geographical range, including locations in western Mexico, Mozambique, and New Caledonia.

Vokes (1970) originally considered *Pazinotus* as morphologically intermediate between *Paziella* Jousseaume, 1880 and *Pterynotus* Swainson, 1883, two other muricid taxa. Fair (1976), Houart (1991a), and Vokes (1992, 1996) kept *Pazinotus* at the subgeneric level, although Radwin and D'Attilio (1976) had elevated it to the generic level, a proposal followed by Houart (1991b, 2006), Leal (1991), and Houart and Héros (2008).

The position of *Pazinotus* in the family Muricidae was revised by Radwin and D'Attilio (1976) who included it in an

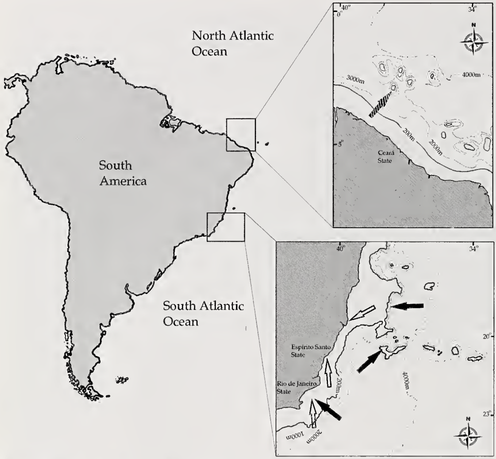

Figure 7. Map of occurrence of *Pazinotus* species in Brazilian coast. Black arrows: *Pazinotus stimpsonii*; dashed arrow: *Pazinotus gili*; white arrows: *Pazinotus bodarti*.

uncertain position in the Muricinae, in a heterogeneous assemblage that also included *Paziella* and *Poirieria*, among others, and stated that further study would be necessary before the subfamilial definition could be established. This subfamilial allocation was also adopted by Vokes (1992). However, Houart (1991b) has suggested that *Pazinotus* should be included in Muricopsinae, based on the radular morphology of *Pazinotus spectabilis* Houart, 1991 and on similarities between *Pazinotus* species from Indo-West Pacific and the Muricopsinae genus *Pygmaepterys* Vokes, 1978. Houart and Héros (2008) confirmed this classification, after the study of radulae of the type species *Pazinotus stimpsonii* and of *Pazinotus falcatiformis*. In the three species above, the rachidian tooth has five prominent cusps.

Despite possible intergradations between the genera that, according to Ponder (1972, 1998) and Kool (1993), make it difficult to adopt a subfamilial classification, we will follow the allocation to Muricopsinae proposed by Houart and Héros (2008). The radulae of both *Pazinotus bodarti* (Fig. 4C) and *P. gili* (Figs. 6D–E) are in the same pattern as those of *P. stimpsonii*, *P. falcatiformis*, and *P. spectabilis*.

The diagnosis herein presented for *Pazinotus* is based mainly on the contributions by Radwin and D'Attilio (1976) and Houart (1991a, b). We included details of the microsculpture of both protoconch and teleoconch and the presence of intritacalx, based on *Pazinotus gili*, the first time such shell layer is described for *Pazinotus*, although it was recorded in *Poirieria* by D'Attilio and Radwin (1971). D'Attilio and Radwin (1971) described the intritacalx as a flat, white, soft and in many cases with intricate sculpture. In *P. stimpsonii* and *P. bodarti*, the intritacalx is not present.

The winglike varices, which produce recurved spines is less marked in *Pazinotus gili*, although it is still easily visible on the outer periphery of the aperture (Fig. 5E). The spiral ornamentation varies considerably in the species (Table 1). It is well marked in *P. bodarti* and *P. goesi* (in this latter species, it is formed of primary, secondary, and tertiary cords), less visible in *P. stimpsonii*, and absent in *P. gili*. The same can be said about the inner denticles of the outer lip (Table 1), which are well marked in *P. stimpsonii* (Figs. 1A, D; 2E–F, H), *P. bodarti* (Figs. 3A, D–E), and *P. goesi*, but weaker in *P. gili* (Figs. 5A, E).

Other species outside of the Atlantic Ocean that are referred to *Pazinotus* show some variation in some aspects of the above diagnosis (Table 1). *Pazinotus spectabilis* Houart, 1991, from New Caledonia has very long and pronounced recurved spines; *Pazinotus advenus* Poorman, 1980 is more similar to *P. bodarti*, especially in the moderately prominent winglike varices.

The protoconch shape and dimensions are virtually identical in the three species from Brazil, but we have no data on the sculpture of protoconch of *Pazinotus goesi* (Table 1). Actually, all descriptions up to now have described the surface of the *Pazinotus* protoconch as smooth. The dense aggregation of microgranules on the last half of the protoconch is easily visible in *P. gili* (Figs. 5I–J) and *P. bodarti* (Figs. 3I–J). However, in *P. stimpsonii* the eroded state of the paralectotype protoconch prevents detailed observation, although some granules can be seen (Figs. 1H–I). Despite its eroded state, it seems that the granules are less dense in the type species. A similar kind of sculpture was found in *Leptotrophon atlanticus* Pimenta, Couto and Costa, 2008 (Pimenta *et al.* 2008: figs. 24–26).

Table 1. Comparative table of western Atlantic *Pazinotus* species: shell characters and geographic distribution. Data about *P. goesi* based on Houart (2006).

	Pazinotus gili	*P. stimpsonii*	*P. bodarti*	*P. goesi*
maximum shell length / teleoconch whorls	6.35 mm / 3.5	12.0 mm / 5.0	12.2 mm / 5	10.8 mm / 4.25
teleoconch microsculpture	one row of pitted lines, with pitted interspaces	one row of pitted lines, with pitted interspaces	two row of pitted lines, with smooth interspaces	unknown
intritacalx	present	absent	absent	absent
protoconch	paucispiral, about 1.5 whorls, globose	paucispiral, about 1.5 whorls, globose	paucispiral, about 1.5 whorls, globose	paucispiral, about 1.5 whorls, globose
protoconch microsculpture	irregular blotches of microgranules closer to the suture, becoming dense and somewhat arranged in spiral rows in the last whorl	apparently smooth, with sparse microgranules well spaced.	irregular blotches of microgranules closer to the suture, becoming dense and somewhat arranged in spiral rows in the last whorl	unknown
denticles on outer lip	three very small and weak denticles on anterior most region (D3–D5)	five strong denticles (ID, D1, D3–D5), D2 absent or hardly discernible	six strong denticles (ID, D1–D5)	five weak, broad denticles (D1–D5)
denticles on the columella	a single, small node	two or three small nodes	a single, small node	two small nodes
spiral sculpture (on last whorl)	one or two very tiny primary cord	four primary cords; secondary cords very tiny, hardly discernable	four primary cords; secondary cords present in at least one shell as very tiny cords	six primary cords; six secondary cords; two tertiary cords
microscopic spiral sculpture	spiral series of pitted lines, space between each pitted line covered by micro holes	spiral series of pitted lines, space between each pitted line covered by micro holes	spiral series of double pitted lines, space between each double pitted line smooth	unknown
varices / spines	five varices on last whorl forming short grooved spines slightly recurved backwards	four strong lamellate varices on last whorl forming a moderately long grooved spines recurved backwards	six strong lamellate varices on last whorl forming very short canaliculated spines recurved backwards	four low, weakly spinose varices on last whorl
geographic range	northeast coast of Brazil	Florida to Barbados; east Panama; Virgin Islands; southeast coast of Brazil	southeast coast of Brazil	Virgin Islands
bathymetry	up to 260 m	up to 200 m	up to 98 m	up to 580 m

The teleoconch microsculpture is also very similar in the three species studied, consisting of spiral series of pitted lines (Figs. 1E–G; 3F–H; 5F–H) (Table 1).

The genus *Pazinotus* is known to occur in the Indo-Pacific (Houart and Héros 2008), New Caledonia (Houart 1991b), and the western Atlantic. In Brazil, *P. bodarti* is restricted to shallow depths off the southeast coast, and *P. stimpsonii* was recorded from deeper waters, also off the southeast coast. *Pazinotus gili* new species, on the other hand, is up to now known only from the Canopus Bank, at about 260 m depth off the northeast coast (Fig. 7).

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Morphology of *Elimia livescens* (Mollusca: Pleuroceridae) in Indiana, U.S.A. covaries with environmental variation

Ashley Dunithan*, Stephen Jacquemin, and Mark Pyron

Aquatic Biology and Fisheries Center, Department of Biology, Ball State University Muncie, Indiana 47306, U.S.A.

Correspondence, Mark Pyron: mpyron@bsu.edu

Abstract: We examined covariation of morphology with local and regional environmental variables in the widespread North American freshwater gastropod *Elimia livescens* (Menke, 1830). Geometric morphometrics was used to quantify shape of individuals collected at sites in Indiana, U.S.A. We used the Procrustes superimposition method and relative warp analysis to examine variation among individuals. We found that shape of *E. livescens* covaried significantly with flowing versus non-flowing habitats, drainage area, latitude and longitude, water temperature, conductivity, substrate type, and the presence of woody debris. Individuals with smaller apertures and streamlined shape occurred in smaller watersheds in the southeastern regions of Indiana, and in local habitats with low water flow, low conductivity, large, coarse substrate (rip-rap), low frequency of woody debris, and higher dissolved oxygen. We suggest that morphological variation is an integral part in maintaining a wide distribution and maximizing local success for an aquatic gastropod that occurs in a variety of environments.

Key words: morphological variation, freshwater gastropod

Aquatic organisms have morphological variation that can be a response to competition, predation, or habitat variation (Abrams and Matsuda 1993, Haase 2003, Hendry *et al.* 2006). Variation in phenotypes with environmental conditions is likely a response by organisms that results in increased survival and persistence across a wide range of habitat types (Wainwright and Reilly 1994). The identification of intraspecific morphological variation across multiple environments provides evidence for adaptive strategies that influence the current diversity of forms.

Morphology of gastropods varies with multiple environmental and biotic variables (Covich 2010). For example, morphological variation in gastropods can result from predation pressure (Vermeij 1993, Holomuzki and Biggs 2006), habitat differences (Hollander *et al.* 2006), or population density (Kemp and Bertness 1984). In addition, freshwater gastropods can have extremely plastic morphology (Dewitt *et al.* 2000). Freshwater habitats tend to have higher spatial and temporal variation of attributes such as water chemistry, water temperature, and drying of habitats, compared to marine habitats (Cole 1994). This physical variation results in high potential shape variation among populations of freshwater gastropods.

Morphometrics is the study of shape variation among individuals (Bookstein 1991, Dryden and Mardia 1998). Geometric morphometrics is a quantitative approach to analyze shape using methods that compare information about relative spatial configurations of shape, typically arranged as predefined landmarks, against an average configuration (Zelditch *et al.* 2004). The geometric morphometric approach provides advantages over traditional truss-based analyses (Wainwright and Reilly 1994, Zelditch *et al.* 2004). Traditional morphology truss-based methods are limited in providing information about the geometric structure of an object and require complex amendments to analyses to account for variation in size (other than for ontogeny). Morphometrics applies the concept of Kendall's shape space to improve traditional morphological analyses through application of scale that is used to identify differences in shape between two configurations (Zelditch *et al.* 2004). Geometric morphometrics has been used for a wide array of topics including biogeographic comparisons, intraspecific variation and phylogeography (Rosenberg 2002, Hayes *et al.* 2006, Hendry *et al.* 2006). Parsons (1997) found high variation in shell morphology of marine gastropods across geographic ranges. Dillon (1984) and Haase (2003) examined morphometric variation in freshwater gastropods for systematics and ecological hypotheses. However, relatively little research has been conducted on variation in morphology in freshwater gastropods as a response to local and regional environmental variables.

In this study we use geometric morphometrics to quantify morphologic variation in an abundant freshwater pleurocerid gastropod *Elimia livescens* (Menke, 1830). Although multiple biotic and environmental variables likely influence the morphology of *E. livescens*, we tested the hypothesis that several local habitat and regional environmental variables have significant correlations with morphology. The objective of this study was to use geometric morphometrics to visualize shape variation among populations of *E. livescens* in Indiana watersheds and to test for relationships with environmental variables.

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* Current address: University of Texas at Tyler, Department of Biology, Tyler, Texas 75799, U.S.A.

MATERIALS AND METHODS

Elimia livescens were collected at sites in Indiana from 2006–2008 during the summer (Jun–Aug) in a survey by Pyron *et al.* (2008). Our current analysis included only sites with at least three individuals collected (39 sites, Fig. 1). All available habitats were sampled by hand collection on woody debris, undersides of stones, and aquatic vegetation. Collection durations were one person-hour per site. Gastropod samples were preserved in 70% ethanol for sorting and identification in the laboratory. Environmental parameters collected at each site included presence of water flow, dissolved oxygen, water temperature, conductivity, pH, substrate composition, latitude, and longitude. Water chemistry measurements were collected using a portable Quanta Hydrolab, GPS coordinates were calculated using a Garmin GPS 60 handheld unit, and substrate was estimated visually into dominant categories (Pyron *et al.* 2008).

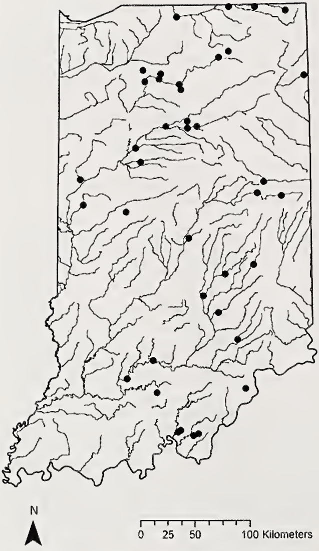

Figure 1. Location of sites. Indiana, U.S.A.

Collections of gastropods with shells that were not broken were spread on a grid and individuals selected using a random number generator. We used a Nikon D70 digital camera mounted on a Zeiss dissecting microscope to capture images. Individuals were positioned with the apex facing upward and adjacent to a scale in mm. Individual orientation was controlled through the use of a common platform that oriented each individual identically. Only adult individuals of similar size (± 3 mm) were included in the analysis to reduce effects of allometry. Twelve landmarks (Fig. 2) were digitized using tpsDig ver. 2.11 (Rohlf 2008). Landmarks included columella, origin of body whorl, widest margin of body whorl, second suture of spire, shell apex, aperture origin, widest margin of aperture lip, junction of upper and lower lip, and median of first suture.

Individuals were rotated, scaled, and translated using the Procrustes, or general least squares (GLS) method (Rohlf 1990). This removes body size as a covariate of shape and generates a reference shape configuration based on all sampled individuals (Zelditch *et al.* 2004). We used relative warp analysis (RWA, Bookstein 1991) to quantify shape variation among individuals using tpsRelw ver. 1.45 (Rohlf 2007). RWA compares relative landmark placement in a given individual to a reference configuration of all individuals. This method computes a principal components analysis (PCA) of the covariance matrix generated from the scaled

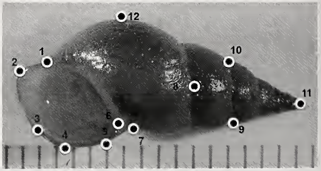

Figure 2. Locations of 12 landmarks on an individual *Elimia livescens*. Scale is in mm. Landmarks denote columella, origin of body whorl, widest margin of body whorl, second suture of spire, shell apex, aperture origin, widest margin of aperture lip, junction of upper and lower lip, and median of first suture.

individuals (Bookstein 1991). We used the default program settings: alpha = α, Bookstein component, and $\lambda = 0$. To reduce effects of variation among the landmarks we selected that fell along curvatures we used semi-sliding landmarks for the following landmarks: LM 3–2/4; LM 5–4/7. The resulting shape variation was explained by a series of relative warps, or multivariate axes, that were visualized using thin-plate splines (deformation grids). Axes represent the maximum amount of variation and are interpreted through eigenvalues and percent variation explained (Zelditch *et al.* 2004). Resulting axes were tested for Pearson's correlations with environmental variables using Minitab software (ver. 15). To avoid Type I error inherent in multiple comparison testing we used the QValue methodology (Storey 2002) to control false discovery rate. We estimated false discovery rates using QVALUE ver. 1 software (Storey 2002) for R (Ihaka and Gentleman 1996). Q values were interpreted identical to *p* values (i.e. alpha level). However, they were not as conservative as traditional Bonferonni corrections. Q values were calculated using all correlations coefficients and created a minimum estimate of false discovery across multiple correlations. Alpha was set at 0.05 for all tests of significance.

RESULTS

We analyzed images of a subset of 191 individual *Elimia livescens* from 39 sites (31 streams and 8 lakes) for morphological analyses (Fig. 1). Site abundance at the 39 sites varied from 3 to 1,441 individuals with a mean of 258 (*SD* = 319). Mean (± *SD*) dissolved oxygen was 8.2 (± 1.8) mg/l, mean water temperature was 22.8 (± 3.7) °C, mean conductivity was 510 (± 135) µmhos, and mean pH was 8.2 (± 0.5). Fine substrates (sand and silt) were the dominant substrate category and occurred at 60% of sampled sites. Gravel and cobble substrates occurred at 50% of sites. Rip-rap, boulder, and bedrock occurred at 15% of sites. Submerged vegetation and woody debris were observed at 30% of the sites.

Our image sample size for each site ranged from 3–5 with a mean of 4.8 individuals (SD = 0.9). The RWA resulted in three significant axes that explained 42%, 17%, and 11%, respectively, of the variation among individuals (Fig. 3). Relative warp (RW) one explained variation in individuals that loaded positive with a decrease in aperture width, shortening in body whorl, and an elongation of the apex length (Fig. 3). Individuals that loaded positively on this axis tended to occur in streams located in southeastern Indiana (Ohio River drainage), in small drainage subbasins with higher dissolved oxygen, lower conductivity, coarse and less complex substratum (i.e., rip-rap, Table 1). Individuals on this axis were differentiated by habitats with and without water flow. Individuals that loaded positively on this axis tended to occur more frequently in lake habitats (2-sample t-test, $t_{63} = 2.7$, $P < 0.01$).

RW2 axis explained variation in apex length, body whorl length, and aperture width (Fig. 3). Individuals that loaded positively on this axis tended to have a robust and thick overall appearance (shortened and wider shell). Individuals that loaded positively on this axis occurred in streams with higher water temperature and lower frequencies of coarse substrate (gravel/cobble, Table 2). RW3 explained variation in overall body width relative to length. Snails that loaded positively on this axis tended to have a longer and thinner spire, and wider body than the consensus shape. Individuals that loaded positively on this axis occurred in lower temperature streams located in the western portion of Indiana (Table 1).

DISCUSSION

Potential explanations for intraspecific shape variation among sites are directional selection, developmental plasticity, or both (Dillon 1984, Carvajal-Rodríguez *et al.* 2005, Pigliucci 2005, Minton *et al.* 2008). One interpretation is that species with the capacity to change rapidly in response to fluctuating environments will experience greater success than species that require a span of multiple generations for gradual phenotypic selection. Phenotypic plasticity provides species the ability to tolerate high environmental variation, and the result is typically widespread success. However plasticity is not independent of the genetics of an organism and can be an additional selective character (Via *et al.* 1995).

Identification of morphological correlates with environmental variation provides evidence of morphological variation as a potential adaptation that maximizes success in those environments. We found differences in aperture width and shell elongation for *Elimia livescens* that were collected from lake and stream habitats, in addition to several other local habitat variables. These results are similar to the results of Minton *et al.* (2008) where gradients in aperture width and shell elongation correlated with river location. Our prediction is that morphology during development is strongly influenced by presence of water flow, water chemistry, and substrate variables. We predict that substrate variation is correlated with gastropod morphology because substrate composition covaries strongly with stream gradient and water flow. Higher gradient streams with increased water flow tend to have larger substrata. Langerhans (2008) described a model for fish development in low-water flow and high-flow environments that explained the results of other fish morphology studies. Rivera (2008) found differences in carapace shape that varied with water flow regime (lake vs. stream) environments in a turtle species. The variation that Rivera (2008) identified for turtles in flowing habitats provided a hydraulic improvement in higher

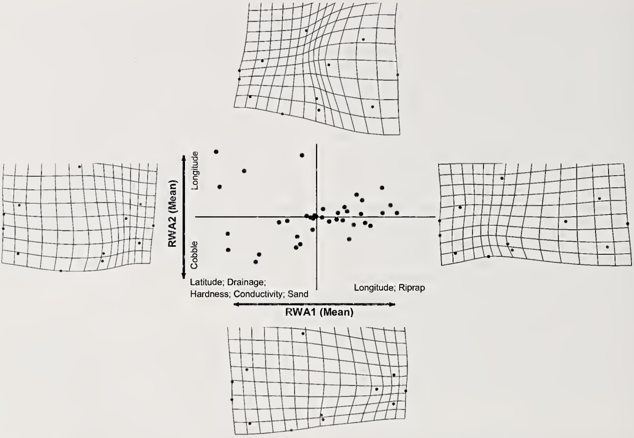

Figure 3. Relative warp analysis axes 1 and 2 mean site loadings. Deformation grids represent individuals at axis extremes. Significant environmental correlations are labeled.

water flows. Similar patterns of shell variation with water flow hydraulics are potentially present in *Elimia*. Water chemistry may further influence developmental morphology of *E. livescens* through impacts on growth based on availability of calcium carbonate or other ions. Growth rates of other freshwater gastropods vary with water chemistry including calcium carbonate (Madsen 1987).

An analysis of the complete gastropod assemblages for Indiana sites (of which our current sites were a subset) resulted in *Elimia livescens* occurring in increased abundances in larger streams with gravel and cobble substrates and lower conductivity (Pyron *et al.* 2009). If individual *E. livescens* complete their life cycle near these increased abundance sites, the habitat variables we measured are likely causes or correlates of the increased abundances. Streams with larger substrata may provide refuge from water flow, protection from predators, and increased foraging habitats.

We found spatial morphological variation that was correlated with environmental variation among gastropod populations, a similar result to populations in other studies. Phenotypic and morphological variation of marine gastropods are responses to long-term climate change (Hellberg *et al.* 2001), salinity and tidal influence (Jorgensen 2002), and upwelling currents and primary productivity (Teusch *et al.* 2002). Dillon (1984) identified morphological variation among populations of *Elimia proxima* that was significantly correlated with river location and water chemistry. Hayes *et al.* (2006) found high morphological variation among sites for *Elimia comalensis* (Pilsbry, 1890). This species is an endemic of several central Texas spring-run streams, and morphological variation was attributed to differences among local environments. Intraspecific variation in gastropod morphology with variation in environments is a common gastropod attribute.

Others have examined morphological variation of gastropods in response to biotic influences such as population density, predation, or parasitic infections. Predation is frequently found to be correlated with gastropod morphological variation. Vermeij and Covich (1978) cited multiple examples of impacts of predation on freshwater gastropod morphology. Shell armament provides protection from predation in New Zealand mudsnails (Holomuzki and Biggs 2006). Holomuzki and Biggs (2006) identified a trade-off between advantages in avoiding predation based on shell armament, and shell smoothness providing an advantage in water flow. Krist (2002) found that morphology of *Elimia livescens* was influenced by the presence of predators and parasite infection. Crayfish had

Table 1. Correlations (Pearson's *r*) for relative warp axes with environmental variables. Significant *q* values (modified *p* values) are in parentheses.

Variable	RWA 1	RWA 2	RWA 3
Drainage area (log 10)	-0.22 (< 0.001)	---	---
Latitude	-0.27 (< 0.001)	---	---
Longitude	0.27 (< 0.001)	0.15 (0.050)	0.22 (<0.001)
Water hardness	-0.24 (< 0.001)	---	-0.20 (<0.001)
Water temperature (°C)	---	---	0.26 (<0.001)
Conductivity	-0.27 (< 0.001)	---	-0.18 (0.010)
DO	0.19 (0.01)	---	0.16 (0.030)
pH	---	---	---
Silt	---	---	---
Sand	-0.21 (< 0.001)	---	---
Gravel	-0.16 (0.010)	---	-0.14 (0.050)
Cobble	---	-0.19 (0.020)	---
Rip-rap	0.21 (0.010)	---	---
Boulder	---	---	---
Bedrock	---	---	---
Wood debris	-0.17 (0.020)	---	---
Submerged vegetation	---	---	---
Eigenvalues	3.2×10^{-3}	1.3×10^{-3}	8.1×10^{-4}

less success feeding on narrow-shelled *E. livescens.* A further example of the influence of a biotic variable is in a marine gastropod, *Littorina littorea* (Linnaeus, 1758), where morphology varies with population density (Kemp and Bertness 1984).

Biotic and abiotic variables affect gastropod morphology. However, the majority of studies of spatial variation in morphology that include potential external biotic or abiotic influences, including ours, provide only correlative analyses and are unable to verify mechanistic causes of morphological variation. The morphological variation we found is likely influenced by additional variables that we did not quantify. Although we did not quantify the presence of predators (Vermeij 1993) and we predict high variation in predators among our sites. Potential predators at these sites include benthic-foraging fishes (e.g., suckers) and crayfish. However, our prediction is that the presence and density of these predators covary similarly with other variables that we measured. The multiple sucker taxa that occur in medium and large streams of the Wabash River watershed are predicted to be absent or in lower abundances in the lakes and smaller streams we sampled based on habitat preferences (Trautman 1986). Crayfish are ubiquitous in the environments we sampled and we predict a crayfish influence on gastropods at all of our sites. A statewide survey of Indiana crayfish (Simon 2001) showed a cosmopolitan distribution of several species of crayfish, including *Orconectes rusticus,* a known predator of freshwater gastropods (Lewis 2001). The presence of parasitic infections that influence morphology of gastropods (Krist 2000) is an additional potential biotic variable that was not measured in our study. Other abiotic variables that have the potential to provide explanation of shape change include river size variation (Vannote *et al.* 1980), disruptions of natural water flow regimes (Poff *et al.* 1997), and additional anthropogenic influences (e.g. pollution, habitat degradation). The majority of streams in the Wabash River watershed have hydrologic alterations (Pyron and Neumann 2008) and other Indiana watersheds have similar hydrologic impacts from agriculture and reservoir release. We recommend further investigation of morphological variation and its implication for conservation.

Genetic analyses may contribute toward confirming whether morphological variation results from directional local selection, phenotypic plasticity, or both in *Elimia livescens.* We hypothesize that genetic variation for phenotypic plasticity in morphology among local populations causes the observed patterns in Indiana *E. livescens* populations. Thus our results are evidence for high intraspecific variation in morphology among populations of an abundant gastropod. The shape variation of *E. livescens* in response to local and regional environmental variation indicates a reason for maintaining biodiversity, conserving ecosystems, and restoring aquatic ecosystems.

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Osilinus lineatus: Incursion and proliferation in Lough Hyne Marine Reserve, SW Ireland

Colin Little[1], Graham M. Pilling[2], Cynthia D. Trowbridge[3,*], and Penny Stirling[1]

[1] Beggars Knoll, Long River Road, Newtown, Westbury BA13 3ED, U.K.

[2] Secretariat of the Pacific Community, B.P. D5, 98848 Noumea Cedex, New Caledonia

[3] Oregon Institute of Marine Biology, P.O. Box 5389, Charleston, Oregon 97420, U.S.A.

Correspondence, C. D. Trowbridge: cdt@uoregon.edu

Abstract: Changes in the distributional ranges of marine indicator species have been used to support hypotheses of climate change on NE Atlantic shores. One such indicator species, *Osilinus lineatus* (da Costa, 1778) (a trochid gastropod), recently colonized the Lough Hyne Marine Reserve in SW Ireland and spread around the lough shores in the last two decades. The incursion and local spread of *O. lineatus* was documented during annual intertidal community surveys of the lough from 1994 to 2010. This species is presently an abundant member of the lough's biota. Although the tidal rapids connecting the lough to the open ocean may have acted as a partial barrier to incursion, substantial populations of the trochid have been noted within the lough from 2003 to the present. Climate change may have enabled this primarily Lusitanian species to penetrate and flourish in the lough. However, based on environmental variables measured and other species surveyed, changes in the semi-enclosed lough may be more complex.

Key words: Trochidae, Gastropoda, population structure, age, longevity

In the northeast Atlantic, many marine species reach either their southern or northern limits around the coasts of Britain and Ireland, which form partial but shifting biogeographic boundaries (*e.g.*, examples in Lewis 1964). Those presently at their northern limits may be expected to move further north if coastal waters continue to warm, as they have done in the last few decades. In SW Ireland, the fully marine sea lough known as Lough Hyne (or Lough Ine) has long been known to contain a mixture of northern boreal and southern Lusitanian species (Bell and Shaw 2002). For example, the lough contains numerous species of Mediterranean fishes, seaweed, and opisthobranchs (with no intervening locations), despite its geographic distance from the Mediterranean Sea (Fig. 1A). If climate warming allowed some species to spread north, it might be expected that the proportion of Lusitanian species in Lough Hyne would rise.

Lewis (1964), Hiscock *et al.* (2004), and many other researchers (reviewed in Crothers 2001) listed "southern" marine species not currently recorded in the northern parts of Britain and Ireland, but which may spread there. Included in this list of indicator species is the trochid gastropod *Osilinus lineatus* (da Costa, 1778) (previously *Monodonta lineata*). The current distribution of *O. lineatus* extends along the west, south, and parts of the east coasts of Ireland, and includes western Wales and the southwestern coasts of England (Crisp 1989, Crothers 2001, Hawthorne and Wiffen 2007, Mieszkowska *et al.* 2007). The reported southern limit is Morocco (Crothers 2001).

Populations of *Osilinus lineatus* in the northern parts of its range were reduced or eliminated after the unusually cold winter of 1962–1963 (*e.g.*, Daguzan 1991). Crisp (1989: 286) reported that "recent survey data" of the Irish coast did not differ "to any appreciable extent from the 1954 data." The geographic northern limit of this species corresponds to ocean fronts, where separate water masses meet (Crisp 1989); such fronts are barriers to planktonic larval dispersal and benthic recruitment. Snail distributions are determined by the interface of water masses.

Subsequent surveys in 2001–2003 (Mieszkowska *et al.* 2005, 2006, 2007) and 2004 (Hawthorne and Wiffen 2007) showed that the snail had recolonized locations close to its former northern limits in Wales, and had spread 50 km east of its former position on the south coast of England. In fact, there are reports of the snail's existence on the continental side of the English Channel, as far northeast as the Netherlands (*e.g.*, Titselaar and Mulder 2007). In Northern Ireland, Nunn (2004, 2006) reported the snail as far north as County Antrim. Furthermore, McGrath and Nunn (2002) suggested that the species used to occur on Clare Island, County Mayo, Ireland; they hypothesized the species became extinct and subsequently

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[*] Mailing address: P.O. Box 1995, Newport, Oregon 97365, U.S.A.

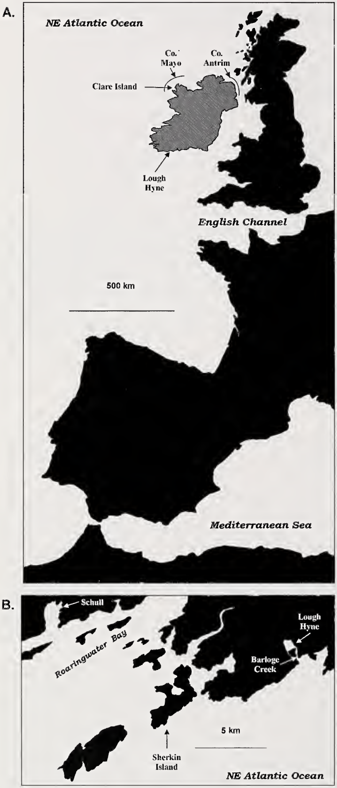

Figure 1. **A**, Map indicating the location of Lough Hyne, County Cork, Ireland relative to the Mediterranean Sea, given that the lough contains many Mediterranean species not commonly found on NE Atlantic shores. **B**, Map showing the location of Lough Hyne relative to the two study sites outside the lough (Schull and Barloge Creek) and to the site of meteorological data (Sherkin Island).

recolonized the island in recent times. Simkanin *et al.* (2005) found that its abundance did not vary between two survey dates (1958 and 2003) on Irish shores but did not present any data by location so it is not possible to determine whether the snails were in the area immediately outside Lough Hyne in 2003. *Osilinus lineatus* was recorded from one site on Sherkin Island (Fig. 1B, *ca.* 10 km from the lough) in 1981 and occurred there at low levels during 1981–2000 (Bishop 2003).

Concurrent to but independent of work by Mieszkowska *et al.* (2005, 2006, 2007) and Simkanin *et al.* (2005) on British and Irish shores, we investigated the incursion and population expansion of *Osilinus lineatus* in Lough Hyne Marine Reserve in the Republic of Ireland (Fig. 1). As part of a long-term monitoring program of the marine reserve, we traced the arrival and proliferation of *O. lineatus* in the lough. A few specimens were seen inside the lough in the period 1976–1984 (Nunn *et al.* 2006) and we recorded one specimen in 1996, but there are no records of the trochid from 1997 until its rediscovery in 2003 (Nunn 2005 and *this study*).

Our objective was to document the population dynamics of *Osilinus lineatus*, which penetrated the tidal rapids to enter the lough somewhat after the species' low-density appearance on the surrounding open-coast shoreline. We compared the population structure of the gastropod at four locations, two inside and two outside the lough, to document the differential ages and growth rates of *O. lineatus*.

MATERIALS AND METHODS

Study region

Lough Hyne Marine Reserve (51°30′N, 9°17′W) is a semi-enclosed, fully marine sea lough in County Cork, southwest Ireland (Fig. 1). The lough (Fig. 2A) is *ca.* 1 km long, 0.5 km wide, and connected to the Atlantic Ocean by a shallow, narrow (25 m wide at high tide, 12 m at low tide) channel called the Rapids. Because of the very narrow entrance with a shallow sill, the lough has a much reduced tidal range: *ca.* 1 m inside and *ca.* 3.5 m outside. The complex nature of the tides in the lough has been well documented (Bassindale *et al.* 1948). The tide ebbs for 8.5 h to low tide; water flows into the lough much faster than it leaves, with a high tide about 4 h after low. Comparable asymmetrical tidal patterns occur in many European sea loughs/lochs/fjords. There are, consequently, no recorded values for tidal levels (such as MHWN [Mean High Water Neaps]) as they are site specific to the lough; thus, we report snail distributions in terms of biological indicator species (*e.g.*, barnacles) that are well recognized on European shores.

The shoreline inside the lough is subdivided into 108 permanently marked sectors (delineated by Renouf 1931) to designate different shore habitats; these sectors, variable in

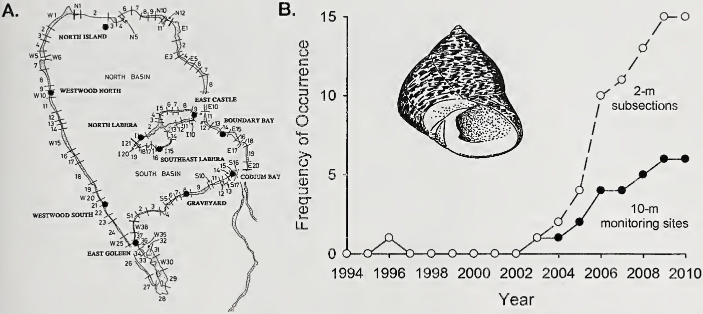

Figure 2. A, Our 10 permanent monitoring sites are indicated with black circles and site names. Renouf sectors, designating similar habitats, are indicated along each shore (108 total within lough). **B**, Frequency of occurrence of *Osilinus lineatus* in Lough Hyne. Solid symbols and lines represent frequency data based on 10 monitoring sites, each of which is 10 m wide on the shore. Open symbols and dashed lines represent data in 2-m subsections of each 10-m wide site (5 subsections/site × 10 sites = 50).

size (as topography varies), are shown (Fig. 2A). *Osilinus lineatus* specimens were sampled in the lough, particularly on the eastern shore (Renouf sectors E1/2 and E10), and at two sites outside the lough: Barloge Creek along the tidal channel connecting the lough to the sea and Schull in Roaringwater Bay to the west of the lough (Fig. 1B).

Study species

Osilinus lineatus is a large, active, trochid snail that attains shell heights of *ca.* 35 mm (Crothers 2001). The snail has been well studied throughout much of its NE Atlantic range (reviewed by Crothers 2001). It occurs throughout much of the intertidal zone but is particularly abundant on shingle- to boulder-strewn shores (Crothers 2001) compared to bedrock-types of rocky shores. The species has lecithotrophic veliger larvae that settle after a few days in the plankton; thus, dispersal is fairly limited. Recruits (>1 mm) prefer cryptic microhabitats such as the undersides of rocks; juveniles move to more visible locations as the snails grow larger (Crothers 2001). The species feeds primarily on microalgae and biofilms. Longevity varies by location but ranges from low estimates of 2.5 years (Daguzan 1991) and 4 years (Regis 1972) to high estimates of 10+ (Regis 1972, Kendall *et al.* 1987) and 15+ years (Crothers 2001).

Population distribution and abundance

Initial awareness of *Osilinus lineatus* in the marine reserve came from our long-term annual monitoring of the benthic communities at fixed sites around the lough—a continuation of earlier studies by Ebling *et al.* (1960) and Little *et al.* (1992). Some preliminary observations were made in 1990 and 1991, but routine surveys were carried out in the last week of August and first two weeks of September each year from 1994 to 2010. At the 10 fixed monitoring sites (black circles in Fig. 2A), we examined 10-m sections of shore at low tide and recorded abundance of ecologically significant species. These stations (Fig. 2A) were a subset of the 20 described by Ebling *et al.* (1960). *Osilinus lineatus* counts were recorded within five 2-m wide subsections at each of 10 sites.

In addition, we conducted visual surveys by walking around the shoreline of the lough, recording the presence or absence of *Osilinus lineatus* visible at low tide in each Renouf sector. Quantitative sampling was conducted in Renouf sector E10 by counting snail densities within 50 quadrats (0.25 m^2) within the shore zone inhabited by *O. lineatus*.

Population structure

To evaluate when *Osilinus lineatus* appeared within the lough, the population structure was investigated by measuring shell heights of all individuals found within specific areas and by estimating ages. Shell height of all *O. lineatus* found within four areas of shore (E10 and E1/2 on the eastern shore within the lough; Barloge Bay and Schull outside the lough, Fig. 1B, 2A) was measured using calipers accurate to the nearest 0.1 mm. Although some researchers have measured snail size as basal diameter (*e.g.*, Williams 1965, Daguzan 1991, Mieszkowska *et al.* 2007), Crothers (2001) and others have favored shell height (also called length) for this species; still others have measured the growth increment as a measure of size (*e.g.*, Mannino *et al.* 2008). Similar variation in methodology has occurred in studies of other topshells around the world (*e.g.*, Red Sea: Ismail and Elkarmi 2006; Mediterranean: Mannino *et al.* 2008; Japan: Nakano and Nagoshi 1984, Takada 1995, 1996, Iijima 2001; China: Wang *et al.* 1995).

Age structure was examined by counting growth-check rings on the shell (Williamson and Kendall 1981, Clarke *et al.* 1990, Crothers 2001). Within the lough this was relatively easy to determine for shells of up to 3 years old, but with adult shells and some of those examined outside the lough, growth-check rings were hard to distinguish, and estimated ages were thus only approximate. We assumed that the first growth-check ring (formed in the first winter when the shells were of the order of 6–7 mm high; Fretter and Graham 1977) was obscured by subsequent rapid growth. Therefore, we added 1 year to the total age of each individual, in addition to the age estimated by growth-check rings. This conservative approach (discussed by Crothers 2001) does not alter the slope of the calculated lines to determine longevity.

Using the calculated age structures, maximum age and mortality rates were estimated, using "catch curves" (*e.g.*, Sparre and Venema 1998) within Microsoft Excel®. This standard approach, which assumes equilibrium conditions and hence constant mortality, assumes that sampled numbers are proportional to the abundance of an age class. The greater the mortality rate, the steeper the decline in the number of individuals found with increasing age. Maximum age and total mortality can therefore be estimated through the regression of the natural logarithm of the frequency of snails vs. a given age. On one hand, the assumption of equilibrium would not be met in an expanding population. On the other hand, two researchers (Stanbury 1975, Crothers 2001) have found fairly even rates of mortality (except during severe climatic events) so the analysis may be robust.

Climatic variables

To evaluate whether changes in *Osilinus lineatus* were indeed directly related to local or regional climatic changes, we evaluated three types of data. (1) Air temperature was obtained from the Irish Meteorological Service (Met Éireann) from three local stations (Cork Airport, Roches Point, and Sherkin Island) near Lough Hyne in August/September of each year. Sherkin Island was considered to be most comparable to the lough based on local conditions. We compared data from two temporal scales: 1959 to 2009 (full data set) and 1994 to 2009 (time during present study). (2) We measured the local lough surface temperature multiple times during each 2-week survey. Early measurements were made at the Kitching Lab's quay in the South Basin (S4/5) whereas from 2001 to 2009 temperatures were measured from the promontory on the North Shore (N11). (3) Local and regional sea surface temperature (SST) data were obtained from the ICES (International Council for the Exploration of the Sea) website. The local SSTs were obtained from the 5° latitude × longitude square directly offshore of the lough from the ICES dataset (http://www.ices.dk/ocean/data/ surface/surface.htm)]; the regional SSTs were obtained from the 5° latitude × 10° longitude square offshore of the Republic of Ireland from the ICES dataset. Pearson correlation analyses were conducted to establish if there were temporal trends in the variables and if trochid appearance and/or abundance were correlated with temperature (seawater or air).

RESULTS

Population distribution and abundance

Osilinus lineatus distribution extended from the level of low water within the lough up to a height of *ca.* 1 m above low water. The top of its tidal range was at about the level of high water of normal spring tides (approx. the top of the barnacle zone). Within the tidal range occupied by *O. lineatus*, the accompanying algal cover varied from almost zero on angular shingle to sparse coverage by *Fucus vesiculosus*. The most frequently associated fauna were the snails *Littorina littorea* (Linnaeus, 1758) and *L. saxatilis* (Olivi, 1792). On the algae, there were other littorines, but *O. lineatus* was always found on rocky substrata.

One specimen of *Osilinus lineatus* was recorded in 1996 at Graveyard on the south shore of the lough; however, no additional specimens were seen until 2003 (Fig. 2B). The number of monitoring sites and subsections thereof at which *O. lineatus* was found (Fig. 2B) showed a gradual rise from 2003 to 2010. In September 2010, 60% of the sites and 30% of the subsections were presently occupied by the snails, indicating that while the snails were widely distributed, they were not continuously distributed on the shore. The overall distribution of *O. lineatus*, assessed by recording presence or absence in Renouf sectors, showed a highly significant increasing occurrence (Likelihood ratio test, $G = 71.9$, 2 *df*, $P < 0.001$) from 2005 to 2008 (Fig. 3). The snail was found in 52 out of 99 sectors surveyed (53%) in 2005 and 58 out of 90 sectors (64%) in 2006. No observations were made in 2007, but in 2008 it was found in 73 of the 108 sectors (68%).

From our observations of *Osilinus lineatus* on the shoreline, Renouf sector E10 was assessed as the site of maximum density: the sector consisted of scattered small

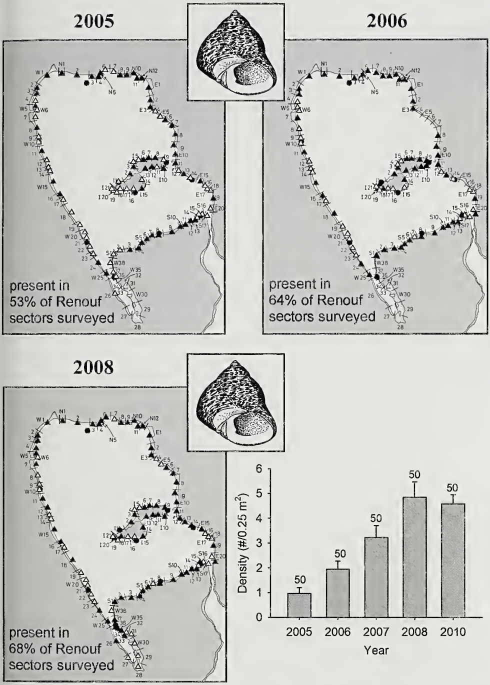
2005
2006
2008
present in 53% of Renouf sectors surveyed
present in 64% of Renouf sectors surveyed
present in 68% of Renouf sectors surveyed
N1
N5
N10
N12
E1
E3
E5
E10
E15
E17
E20
S1
S5
S10
S16
S17
W1
W5
W6
W10
W15
W20
W25
W30
W35
W38
I10
I15
I20
I21
Density (#/0.25 m²)
0
1
2
3
4
5
6
50
50
50
50
50
2005
2006
2007
2008
2010
Year

Figure 3. Increase in the distribution of *Osilinus lineatus* around the lough from 2005 to 2008. Black triangles denote presence of the snail in that Renouf sector; hollow triangles denote absence of snails. Black circles indicate location of 10 permanent monitoring sites. Histogram shows density of *Osilinus* in sector E10; error bars indicate standard error and replication is shown.

angular rocks and boulders on a shallow sloping beach. E10 was, therefore, used to quantify temporal changes in snail density. In 2005, mean density within 50 quadrats was 0.96 per 0.25 m^2 (Fig. 3). In the next three years, snail density increased steadily: by 2008, *O. lineatus* density was 4.84 per 0.25 m^2. Snail densities in 2010 were similar to those in 2008 (Fig. 3).

Population structure

Inside the lough

Population size structure was recorded in sector E10 for 2005–2010 (Fig. 4). In 2005, the population was dominated by snails with a median shell height of 19–20 mm. In 2006, juveniles with a median shell height of 12–14 mm were prevalent and in 2007, these had grown to form the dominants of median shell height 17–19 mm. In 2008, the dominant size class had shell heights of 20–22 mm, but there was another smaller cohort of smaller snails (9–15 mm) present. In 2009, population structure was not measured. However, there was an unusually large amount of rain that year as well as an extreme cold spell (in December 2009 and January 2010). In September 2010, there were almost no juveniles <15 mm shell height (Fig. 4); whether the physical conditions reduced recruitment and/or killed juveniles is not known.

Age structure, as determined by growth-check rings, provides a clearer picture of the population (Fig. 4) than size alone. In 2006 the juveniles of shell height *ca.* 12–14 mm were in their second year of growth (*i.e.*, no visible growth checks, but one presumed from the winter of 2005/2006—see methods). Shells with a height of 17–20 mm were mostly in their third year (one visible growth check, but a second presumed). Based on cohort analysis using "catch curves" (Table 1), very few shells in sector E10 were older than 7 years in 2006, while in 2007 maximum age was estimated at 9.5 years, and in 2008 at 9.2 years. These estimated ages indicate that *Osilinus lineatus* was present at E10 or environs in the late 1990s.

In another area within the lough, Renouf sectors E1/2, the proportion of larger, older snails was higher (Fig. 5A). These sectors consisted of solid bedrock in some places and vertical dry-stone walls with many crevices in others. In 2006, maximum age was estimated to be 8 years, and in 2007 to be 14+ years (see Table 1). These results indicate that *Osilinus lineatus* was present at E1/2 or environs in the late 1990s.

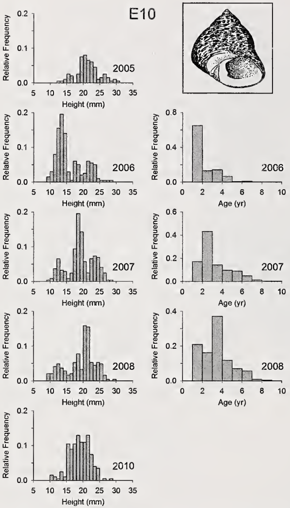

Figure 4. Population size and age structure of *Osilinus lineatus* in Renouf sector E10 of Lough Hyne for the years 2005–2010.

Outside the lough

Immediately outside the lough in Barloge Bay at the seaward end of Barloge Creek (Fig. 1B), where the shore consisted of large boulders and areas of sloping bedrock, the population was mostly made up of large *Osilinus lineatus.* In 2006, no snails smaller than 14 mm shell height were recorded; in 2007, none smaller than 18 mm were found (Fig. 5B). These snails were all at least two years old with a maximum age of 9, based on check rings, and 13 to 15 years, based on cohort analysis (Table 1).

For comparison, a population was also investigated at Schull, 15 km west of Lough Hyne on the open, but sheltered, shore of Roaringwater Bay (Fig. 1B). Here, where the shore consisted of sloping bedrock with ravines, gullies, and rock pools, a wide range of sizes and ages was found. Juveniles in their second year were found next to large adults that appeared to be 9 years old (Fig. 5C), based on check rings, or 12 years old, based on cohort analysis (Table 1). *Osilinus lineatus* was, thus, present outside the lough in the early to mid 1990s.

Inside vs. outside

A nested ANOVA with sites nested within location (inside vs. outside lough) indicated that there were highly significant differences in shell length ($F = 30.0, P < 0.001, N = 781$) and age ($F = 27.0$, $P < 0.001$, $N = 781$) [Note that N denotes sample size; n, the replication, was two sites per location]. On average, Renouf sector E10 (Fig. 2A) had the smallest and youngest trochids (18.3 ± 0.3 mm, 2.7 ± 0.1 years); the other three sites had snails that averaged 2–4 mm longer and 1–1.4 years older than the E10 ones.

Climatic variables

The August and September values (typically the peak monthly mean of the year) for oceanic and lough sea surface temperatures did not show any convincing statistical trend of increase over recent years (Fig. 6) (regional: $P = 0.948$, $r = 0.019$; local: $P = 0.080$, $r = -0.612$; lough: $P = 0.084$, $r = -0.460$). In fact, the relationship tended to decrease, rather than increase, in the past two decades. A similar result was found with the August air temperatures at Sherkin Island in the Met Éireann 1994–2009 data set (maximum: $P = 0.790$, $r = -0.075$; minimum: $P = 0.191$, $r = -0.357$). Furthermore, there was no correlation between snail abundance and sea surface temperature at any spatial scale (P-values ranged from 0.078 to 0.364, with all but one case with $P > 0.100$). Air temperatures did exhibit a warming trend over a much longer (5-decade) timescale (Fig. 7) though only the minimum values were statistically significant ($P < 0.05$ for all three County Cork sites). Finally, seawater and air temperatures both show a great deal of year-to-year fluctuation with the lough SST consistently warmer than the outside SST (Fig. 6A).

DISCUSSION

Appearance in Lough Hyne

Osilinus lineatus typically occurs most frequently and abundantly on unconsolidated rocky shores (shingle, boulders, and cobbles: Crothers 2001, Little *et al.*, pers. obs.). The rocky shore monitoring program has concentrated on Ebling's historical sites with the high and mid shore areas being primarily consolidated rock (bedrock). With the exception of one individual seen in 1996 at Graveyard (Fig. 2A), it was not until *O. lineatus* populations were abundant enough to "spill over" and populate our study habitats that we recorded the species. Thus, while we detected the species in 2003 (the same year in which it was found by Nunn 2005, Nunn *et al.* 2006), age-structure analysis of the populations shows that the gastropod must have been present much earlier than this. At Renouf sector E10, maximum age in 2007 was estimated at 9.5 years. At Renouf sector E1/2, maximum age in 2007 was estimated at 14+ years (Table 1). Lough

Table 1. Estimates of maximum age and total mortality (Z) (with variability) for *Osilinus lineatus*, by location in County Cork, Ireland and year. †, High level of inter-cohort variability noted in dataset.

Location	Year	Max. age (y)	Mean total mortality (Z y^{-1})	2 *SD* (Z)	*N*
E10, Lough Hyne	2006	6.2	0.90	0.50	200
	2007	9.5	0.60	0.26	246
	2008	9.2	0.57	0.28	246
E1/2, Lough Hyne	2006	8.0	0.78	0.18	115
	2007	14.6	0.37	0.07	275
Barloge Bay	2006	15.2	0.26	0.51†	79
	2007	13.3	0.29	0.24†	99
Schull	2007	12.0	0.39	0.09	161

populations were thus established there well before 2000, possibly by the early to mid 1990s.

The earlier presence of *Osilinus lineatus* in the lough—a few seen on Castle Island between 1976 and 1984 (Nunn *et al.* 2006)—could have provided the origins of these populations. With the exception of the single individual recorded by us in 1996 (this study), no *O. lineatus* specimens were seen in the lough from 1990 to 2003 (Little *et al.* 1992, Nunn *et al.* 2006). Thus, the present populations may represent a new origin. Because this species has external fertilization, scattered individuals are unlikely to form a self-sustaining population (Crothers 2001), and the 1976–1984 individuals may have died out without producing offspring.

If populations of *Osilinus lineatus* were regionally present outside the lough (though in low numbers; Bishop 2003), why did the species take so long to appear within the lough? On one hand, the species is not sexually mature until its second year and then has lecithotrophic larvae with limited dispersal. On the other hand, what other factors may constrain the species? Jessopp (2007) examined the larval mortality of various taxa, including molluscs, passing through the tidal rapids connecting Lough Hyne to Barloge Creek and the Atlantic Ocean. He found a high mortality of larvae, including veligers with delicate shells, passing through the shallow, high-velocity rapids with current speeds up to 3 m/s. Furthermore, Jessopp and McAllen (2007) found that complex hydrodynamics may result in some species being imported into the lough, others being exported, and some moving both ways, depending on factors including season, larval type, behavior, etc. However, veliger larvae of *O. lineatus* were not reported in their study. Furthermore, Crothers (2001) reported that, to his knowledge, the larvae have never been caught in plankton samples, suggesting that larvae may remain near the sea floor. Once *O. lineatus* larvae did survive passage through the rapids and the diversity of suspension-feeding invertebrates and planktivorous fishes occurring there, the species was able to establish a viable population and expand around the shoreline of the lough.

Timing of incursion

Osilinus lineatus colonized Lough Hyne and spread around its shores over a very similar time period as another "southern" species, namely the fucalean brown alga *Cystoseira foeniculacea* (Trowbridge *et al.* 2011). When seeking for an explanation of these recent range expansions, we hypothesized that climate change has allowed two primarily Lusitanian species to flourish because average temperatures in North Atlantic waters have increased (see, *e.g.*, Mieszkowska *et al.* 2006, 2007). Yet, our local environmental data (Fig. 6A) did

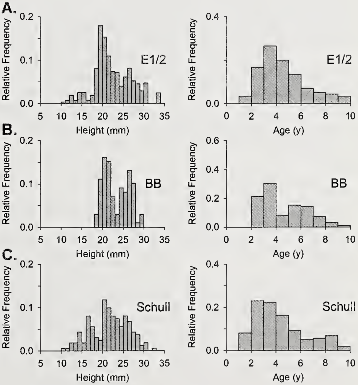

Figure 5. Population size and age structure of *Osilinus lineatus* in 2007. **A**, E1/2 in lough; **B**, Barloge Bay; **C**, Schull, outside lough, County Cork.

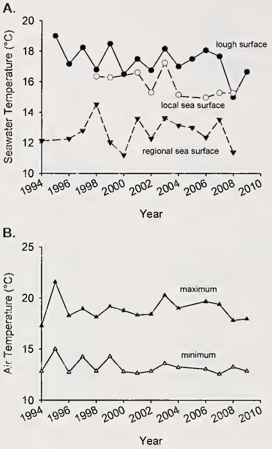

Figure 6. Temporal variation in environmental variables in August/September. **A**, Sea surface temperature within the lough (our data), local SST [5° latitude × longitude square directly offshore of the lough from ICES (International Council for the Exploration of the Sea) dataset (http://www.ices.dk/ocean/data/surface/surface.htm)], and regional SST [5° latitude × 10° longitude square offshore Republic of Ireland from ICES dataset. **B**, Maximum and minimum air temperature from Sherkin Island, County Cork (courtesy of Met Éireann). Air temperatures from Sherkin covaried with those from two other meteorological stations (Cork Airport and Roches Point, County Cork).

not strongly support this hypothesis. In fact, our results (Fig. 6A) support earlier findings by Minchin (1987, 1992) of (1) considerable year-to-year variability and (2) lough temperatures 2.5 to 4.5° C warmer than the seawater outside the lough.

Fauna and flora may be better indicators of change than purely physical measurements: the organisms respond to changes within their own exact microhabitat, and they also integrate effects over not just the whole year but over their entire life spans. If there has been a change in climate, it would thus be expected that other species in Lough Hyne would show changes: while other Lusitanian species would be predicted to increase, boreal species would be predicted to decrease.

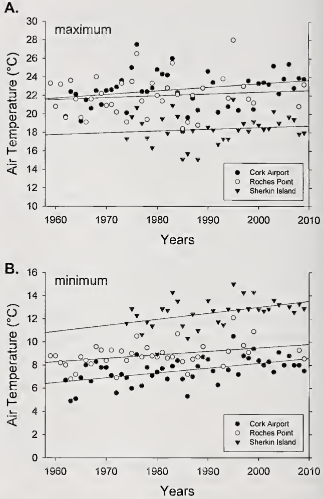

Figure 7. Temporal variation in environmental variables in August/September for the past four decades. Maximum and minimum air temperature from 3 sites near Lough Hyne: Sherkin Island, Cork Airport, and Roches Point, County Cork, Ireland (courtesy of Met Éireann).

Because the lough is for the most part shallow and has a long flushing time (Johnson *et al.* 1995), it is possible that local weather conditions have more control over lough temperatures than ocean temperatures. The asymmetry of the lough's tidal system (Bassindale *et al.* 1948) ensures that much of the intertidal zone is emersed for 8 hours or more in each tidal cycle. If climate change were responsible for some of the population changes in the lough, it could thus act by controlling air temperatures (Hiscock *et al.* 2004), which would directly affect growth and reproduction. *Osilinus lineatus*, which has populations relatively high on the shore, would thus heat up in summer much more than would be supposed by measuring water temperatures.

If the local effects of climate change are to be predicted more effectively, more attention must be paid to the temperature regimes of the microclimates in which biota are found, rather than measuring overall oceanic or offshore temperatures. The studies of mussel temperatures in which temperatures were recorded inside mussel-mimics (Helmuth and Hofmann 2001) serve as an appropriate model. The importance of non-climatic thermal adaptation—for instance the response to solar radiation—has been emphasized by Marshall *et al.* (2010). In terms of following or predicting changes in distribution, most studies have monitored expanding or contracting ranges of indicator species by tracking the arrival or loss of populations along coastlines (*e.g.*, Mieszkowska *et al.* 2006). This is a difficult and time-consuming operation, and we suggest that monitoring semi-isolated bodies of water such as Lough Hyne could offer an alternative way—an advanced indication—of monitoring change. Because isolation allows the effects of warmer temperature to be expressed earlier than in the main body of the sea, bodies such as Lough Hyne could act as early "barometers" or indicators of change. The decline of cold-water kelp (*Saccharina latissima*) in the shallow, partially enclosed waters of southern Norway (Moy *et al.* 2006)—which warm up more rapidly than the open ocean does—may exemplify this approach.

During the period of expansion of *Osilinus lineatus* within the lough, the community-level monitoring surveys by Little *et al.* (pers. obs.) have not indicated major changes in the biota of the intertidal zones with the exception of the incursion of the barnacle *Austrominius modestus* (previously called *Elminius*). The trochid thus appears to be an addition to the fauna, inhabiting its own niche, rather than displacing other species. For example, *Littorina littorea* is still extremely common in sectors where *O. lineatus* has multiplied, and has shown no decline in our routine monitoring sectors. In contrast, there have been major changes in community structure in the shallow subtidal, where the "southern" fucalean *Cystoseira foeniculacea* (Trowbridge *et al.* 2011) and "warm-water" green alga *Codium vermilara* are now common.

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Native Hawaiian succineids prefer non-native ginger (*Hedychium* spp.) plant species in the Kohala Mountains, Hawaii: Conservation ramifications

Wallace M. Meyer III*

Center for Conservation Research and Training, Pacific Biosciences Research Center, University of Hawaii, 3050 Maile Way, Gilmore 408, Honolulu, Hawaii 96822, U.S.A

Correspondence, Wallace M. Meyer III: wmm@email.arizona.edu

Abstract: Although non-native species have been implicated as a major factor in the decline of the native Hawaiian land snail fauna, little attention has been focused on understanding how colonization by non-native plants influences Hawaiian land snail populations. The plant preferences of native Hawaiian succineids in the Kohala Forest Reserve were examined where native plant species and invasive non-native ginger species are present to understand how changes in the understory plant composition may influence succineid populations. Surprisingly, native succineid land snails preferred non-native ginger species to native plant species. This finding suggests that native succineid populations may not be negatively affected when the understory plant assemblage changes from a native plant community to one composed primarily of non-native ginger species. However, this preference also indicates that native succineids may be vulnerable to the effects of ginger control efforts. As such, managers should proceed cautiously with ginger removal efforts if they intend not to harm native succineid populations. Hopefully, future efforts to control non-native ginger species will adapt and minimize any negative effects on native succineid species.

Key words: introduced, invasive, Jacobs' selectivity indices, land snails

The Hawaiian land snail fauna was once exceptionally diverse (more than 750 species) and exhibited extremely high levels of archipelago endemism (more than 99%) (Cowie *et al.* 1995). However, the majority of these unique species are now extinct. Estimates of extinction range from 65–75% (Solem 1990) to as much as 90% (Cowie 2001a, Lydeard *et al.* 2004). Non-native animals have been implicated as a major factor in the decline of the native Hawaiian land snail fauna (Hadfield 1986, Cowie 2001a, Lydeard *et al.* 2004). For example, rapid population declines and extinctions of Hawaiian land snails have been reported following the introduction of both rats and predatory land snails, particularly *Euglandina rosea* (Férussac, 1821) (Burney *et al.* 2001, Cowie 2001b, Meyer and Shiels 2009, Meyer and Cowie 2010). Unfortunately, many non-native plant species have also become established in Hawaii (Smith 1985), but relatively little attention has been focused on understanding how colonization by non-native plants influences Hawaiian land snail populations.

Over 10,000 non-native plants have been introduced to the Hawaiian Islands (Staples and Cowie 2001). Of these, 1,104 species have become established and 86 have become serious pests (Smith 1985, Staples and Cowie 2001). Ginger species *Hedychium gardnerianum* Sheppard ex Ker Gawl, 1824 (kahili ginger), *H. flavescens* Carey ex Roscoe, 1824 (yellow ginger) and *H. coronarium* König, 1783 (white ginger) are well known examples of problematic non-native species (Smith 1985, Daehler *et al.* 2004). These species were purposely introduced to Hawaii from Asia for the ornamental trade, and have subsequently colonized many natural mesic (wet) habitats and shaded or semi-shaded forests throughout the Hawaiian archipelago (Smith 1985). In Hawaiian rainforests, dense monotypic under-stories of ginger are known to restrict regeneration of native plants and facilitate recruitment of other non-native plant species, particularly strawberry guava, *Psidium cattleianum* Sabine, 1822, leading to the formation of a new exotic forest type dominated by a strawberry guava overstory and a ginger understory (Minden *et al.* 2010).

The purpose of this study was to examine the plant preferences (the plants that the snails are found on) of native Hawaiian succineids in areas where native plants and invasive ginger species are present to understand how changes in the understory plant composition may influence extant succineid populations. Certain rainforest succineids seem relatively abundant (Brown *et al.* 2003, 2006, Rundell and Cowie 2003). However, Rundell *et al.* (2004) and Holland and Cowie (2009) suggested that one third to a half of the 42 recognized Hawaiian succineid species may be extinct. In addition, Meyer (2009) described that many of the abundant rainforest succineid species are actually complexes of cryptic species some of which are rare and have limited distributions indicating that they may be deserving of conservation attention. Despite high endemism and conservation value, relatively little attention has been focused on the ecology of the extant Hawaii succineids, with the exception of efforts to understand the life histories of a few species (Brown *et al.* 2003, 2006, Rundell and Cowie 2003). Therefore, it is important to assess aspects of the ecology of these snails now, so that conservation managers can make informed decisions to prevent further population decline and loss of these species, which are representatives of the few remaining Hawaiian land snail species.

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* Present address: Department of Entomology, University of Arizona, 1140 Main Campus Drive, Forbes 410, Tucson, AZ 85721, U.S.A.

MATERIALS AND METHODS

Study Area

The study was conducted in a 112 x 10 m area in the Kohala Forest Reserve located on the north portion of the island of Hawaii (20°03′53″N, 155°40′07″W). It is classified as a wet forest, which is characterized by annual average rainfall > 4,000 mm (Juvik and Juvik 1998). It is also classified as a cloud forest and collects more water than its annual rainfall numbers indicate, because most of the water input into this forest comes from condensation on plants as clouds pass through the forest (John Delay, pers. comm.). The native plant diversity from the Kohala forest mainly consists of plants in the genera: *Metrosideros*, *Broussaisia*, *Melicope*, *Cibotium*, *Ilex*, *Clermontia*, *Cheirodendron*, *Frecycinetia*, *Dicranopteris*, and occasionally *Trematolobelia*. Non-native gingers *Hedychium* spp. have become common in this area, but have yet to form monotypic understories. No *Psidium cattleianum*, strawberry guava, was found at the study site.

Two visibly distinct microhabitats at the site can easily be differentiated. The first, the "shrub-microhabitat", consists of all plants being smaller than chest height including the dominant tree species *Metrosideros polymorpha* Gaudich, 1830. The second, the "canopy-microhabitat", consists of a distinct over-head canopy of *M. polymorpha*, with larger *Cheirodendron* spp., and a ground that is covered by a thick moss. Both microhabitat types had non-native ginger species present.

Succineid Diversity in Study Area

Rundell *et al.* (2004) and Brown *et al.* (2006) reported collecting only *Succinea newcombiana* Garrett, 1857 in their research sites in the Kohala Mountains. However, I found succineids at my study site with two distinct shell morphologies: (1) a dorso-ventrally flattened shell that inhibits the snail from retracting its entire body into the shell and matches the description of *S. newcombiana* and (2) a conical shell shape with enough volume to retract their entire body into the shell. Preliminary molecular analyses have confirmed that the two morphological types are different species and both are native to Hawaii as they fall within a succineid clade that consists entirely of native Hawaiian species (Meyer, unpublished). As such, the dorso-ventrally flattened snail and conical snail are further referred to as *S. newcombiana* and *Succinea* sp., respectively, throughout the rest of the paper. Because a snail's shell shape can correlate with environmental factors and their ecology (Cain and Cowie 1978, Goodfriend 1986) and the two morphological types are different species, I examined the plant preference of snails with both shell morphs independently.

Sampling Design

Two sampling trips were conducted on 02 February 2005 and 11 March 2005. During each sampling trip, twelve 10 m transects were run parallel to one another in one contiguous forest patch. Each transect was separated from the others by 10 m. The sampling site was ~15 m away from the small dirt road to limit edge effects. On the first sampling trip, all plants within 1 m of either side of the twelve transects were identified to genus. On both sampling trips, all plants within 1 m of each transect were examined for succineid snails by searching the entire plant from the ground to eye height (tops and bottom of leaves, stems and trunks were surveyed). If a snail was found it was identified by shell morphology (*S. newcombiana* or *Succinea* sp.), and the plant it was found on was identified to genus. The same transects were examined on each sampling trip.

Analyses

A two-factor ANOVA was used to test for differences in abundance between microhabitats for each succineid species. All data met the assumption of equal variance and normal distribution. The two factors were: (1) a fixed micro-habitat effect (Shrub vs. High Canopy) and (2) a random time effect (two collection dates). T-tests were used to test for differences in plant taxa abundance between the 2 microhabitat types.

To estimate plant preferences of the succineids with different shell morphologies (*S. newcombiana* or *Succinea* sp.), Jacobs' selectivity indices (Jacobs 1974) were calculated using the following formula:

$$D_{ia} = (r_i - p_a) / (r_i + p_a - 2\, r_i\, p_a)$$

where D_{ia} is the selectivity index of snail species *i* for plant type *a*, r_i is the ratio of plant type *a* use to all the other plant types used by that species, and p_a is the ratio of plant *a* to all the other plants available for the individual to use within the local area. If the species preferentially uses a certain plant the D_{ia} score will be positive, if it avoids a plant the D_{ia} score will be negative. The range of potential scores is from 1 to -1. A separate Jacob's selectivity index was calculated for each plant species in each micro-habitat type (canopy and shrub microhabitat).

RESULTS

Over both sampling trips, 67 *S. newcombiana* and 33 *Succinea* sp. were found. Significantly more *S. newcombiana* were found in the high canopy microhabitat and more were collected on the second collection date (Table 1), which was likely the result of high snail activity associated with extremely wet weather. However, there were no differences in number of *Succinea* sp. found in the two micro-habitat types or between collection dates (Table 1).

Succineids were found on native plants in the following genera *Metrosideros*, *Broussaisia*, *Cibotium*, and *Ilex* and on *Hedychium* spp. (invasive gingers). All of these plant genera were found in both microhabitat types, but significantly higher densities of *Hedychium* spp. were found in the canopy habitat (Table 2). *Succinea newcombiana* was not found on *Broussaisia* (Table 3), a plant favored by succineids in the Waiakea Forest Reserve (Brown *et al.* 2003, Meyer 2009).

The plant preferences of *S. newcombiana* and *Succinea* sp. were estimated using the Jacobs' index of selectivity. One *Succinea* sp. was excluded from analyses because it was found on one of the researchers and not on a plant. Non-native gingers (*Hedychium* spp.) were preferred by both *S. newcombiana* and *Succinea* sp. in both micro-habitat types (Table 3). Succineids were only found on gingers in the high canopy microhabitat.

DISCUSSION

Typically, conservation of Hawaiian biodiversity requires reducing or controlling the impact of non-native plant and animal species (Cowie 2004, Burney and Burney 2007). However, this study clearly demonstrates that native succineid land snails in the Kohala Forest Reserve prefer non-native ginger species to native plant species. This preference was extreme in the canopy microhabitat where ginger species are more dense (Tables 2, 3) suggesting that preference for non-native gingers may increase as densities of non-native ginger species increase. This is consistent with a study by Brown *et al.* (2006) that found *S. newcombiana* to be relatively abundant in an area where this understory transformation had already occurred. This result suggests that some native succineid populations may not be deleteriously affected when the understory plant assemblage changes from one dominated by native plants to one consisting primarily of non-native ginger species.

However, presuming succineids will not be deleteriously influenced by non-native ginger colonization from just looking at plant preference data may be short sighted. In Hawaiian rainforests, dense under-stories of ginger can restrict regeneration of the dominate tree species *Metrosideros polymorpha* and facilitate recruitment of a non-native tree species *Psidium cattleianum*, strawberry guava, leading to the formation of a new exotic forest type (Minden *et al.* 2010). To what extent this novel forest type would influence native succineids is unknown. Canopy transformation can influence many ecosystem processes. In this instance, water acquisition from the clouds and retention of water in the system would likely decrease as *P. cattleianum* became more dominant (John K. Delay pers. comm.). Since *S. newcombiana* cannot retract its entire body into its shell, changes in moisture regimes may influence this species activity and potentially its fitness. Brown *et al.* (2006) found that *S. newcombiana* activity was reduced when the humidity was low. As such, it is difficult to rule out if influences of ginger species on canopy formation over time may negatively impact Hawaiian succineids.

Succineid preference for non-native ginger species may also lead to the demise of these native snail populations. Because non-native gingers represent a large threat to maintaining Hawaiian native plant biodiversity, control efforts have been proposed for some rainforest areas on the island of Hawaii and include spraying of herbicide, manual removal, and biocontrol (Anderson and Gardner 1999). Such

Table 1. Density of two succineid species in the two micro-habitat types (Canopy and Shrub) found on the two collection dates. A two-factor ANOVA tested for differences in numbers of individuals between microhabitats and between the two collection dates.

Species Collection Date	Density in Canopy (No./m^2)	Density in Shrub (No./m^2)	Source	*F*	*DF*	*P*-Value
S. newcombiana			Collection Date (CD)	6.16	1.20	0.02
02 February 2005	2.8	0.9	Microhabitat (M)	7.20	1.20	0.01
11 March 2005	7.3	2.5	CD x M	1.36	1.20	0.25
Succinea sp.			Collection Date (CD)	0.52	1.20	0.48
02 February 2005	2.0	1.0	Microhabitat (M)	0.52	1.20	0.48
11 March 2005	1.0	1.3	CD x M	1.45	1.20	0.24

Table 2. Mean density (number of individuals/m^2) of plant taxa that succineids were found on in the two micro-habitat types: shrub and canopy. NS indicates non-significance (*P*-value > 0.05). *P*-values < 0.05 are reported.

Taxa	Density in Canopy Microhabitat (No. /m^2 ± 1 *SD*)	Density in Shrub Microhabitat (No. /m^2 ± 1 *SD*)	T-Test Significance (*P*-value)
Ilex	0.21 ± 0.20	0.39 ± 0.47	NS
Metrosideros	0.60 ± 0.44	1.12 ± 0.83	NS
*Hedychium**	1.05 ± 0.62	0.36 ± 0.42	0.045
Broussaisia	0.30 ± 0.36	0.38 ± 0.60	NS
Cibotium	0.34 ± 0.22	0.14 ± 0.14	NS

* indicates non-native plant genus

efforts will likely intensify as non-native gingers continue to spread into native forests. However, native succineids' preference for non-native ginger species suggest that conservation practitioners should proceed cautiously with management and control efforts that involve non-native ginger species, because some removal strategies (particularly herbicide use and manual removal) of non-native ginger may deleteriously affect native succineid snail populations. My goal in publishing this paper is not to impede removal of ginger species from Hawaiian rainforests. Preference does not indicate that succineid populations would be harmed if non-native gingers were absent and should not be used as an excuse not to remove non-native ginger species from Hawaiian ecosystems. Instead, preference of succineids for non-native gingers simply indicates that a high proportion of succineids in the Kohala Forest Reserve can currently be found on non-native ginger plants. As a result, ginger removal efforts may harm large proportions of native succineid populations. Hopefully, future efforts to control non-native ginger species can adapt and minimize any negative effects on native succineid species.

It is becoming increasingly clear that prudent management of biodiversity requires precautionary and adaptive management approaches (Doak *et al.* 2008). Complex interactions among native and non-native species make it difficult for conservation practitioners to develop strategies to effectively manage natural resources and protect native biodiversity. In the Kohala Forest Reserve conservation practitioners are faced with an interesting dilemma; how to control non-native ginger and not harm native succineid populations? Succineids were probably rarely considered in discussions of ginger control prior to this study. Consequently, malacologists need to focus more efforts on the ecology of the extant land snails (even those that are still abundant) so conservation practitioners can make informed decisions and prevent further population declines and species loss.

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Table 3. Plant selection by succineids in two microhabitats of the Kohala Forest Reserve. Positive and negative values of the Jacobs' selectivity index (D) indicate that different plants were used more or less frequently, respectively, than expected by chance. All succineids found in the canopy microhabitat were found only on non-native ginger plants (*Hedychium* spp.)

	S. newcombiana		***Succinea* sp.**	
Plant Genera	**Canopy**	**Shrub**	**Canopy**	**Shrub**
Ilex	-	-0.28	-	-0.37
Metrosideros	-	-0.39	-	-0.46
*Hedychium**	1	0.61	1	0.47
Broussaisia	-	-	-	0.37
Cibotium	-	-0.25	-	-0.14

* indicates non-native plant genus

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RESEARCH NOTE

Tracking behavior in the snail *Euglandina rosea*: First evidence of preference for endemic vs. biocontrol target pest species in Hawaii

Brenden S. Holland[1], Taylor Chock[2], Alan Lee[3], and Shinji Sugiura[1,4]

[1]Center for Conservation Research and Training, Pacific Biosciences Research Center, University of Hawaii at Manoa, 3050 Maile Way, Gilmore 408, Honolulu, Hawaii 96822, U.S.A.

[2]Whitman College, 345 Boyer Ave., Walla Walla, Washington 99362, U.S.A.

[3]Depauw University, P.O. Box 37, Greencastle, Indiana 46135-0037, U.S.A.

[4]Department of Forest Entomology, Forestry and Forest Products Research Institute (FFPRI), 1 Matsunosato, Tsukuba, Ibaraki 305-8687, Japan

Correspondence, B. S. Holland: bholland@hawaii.edu

Abstract: The predatory snail *Euglandina rosea* (Férussac, 1821) is native to the southeastern U.S.A. and has been widely introduced as an attempted biological control agent for agricultural pest gastropods, often targeting the giant African snail *Achatina fulica* Bowdich, 1822. However, *E. rosea* may impact native land snails rather than *A. fulica*, particularly in Hawaii. Laboratory prey preference trials for *E. rosea* were conducted using three prey taxa, including the endemic endangered Oahu tree snail *Achatinella lila* Pilsbry, 1914, the giant African snail *A. fulica*, and the introduced Asian trampsnail *Bradybaena similaris* (Rang, 1831). Trials were conducted in the laboratory on branches of ohia lehua trees, *Metrosideros polymorpha*, an important habitat component for Hawaiian tree snails. Y-shaped ohia branches were used to simulate tree snail habitat and test *E. rosea*'s ability to track and pursue prey via slime trails on branches, and to evaluate prey choice by offering trails of two different species simultaneously and slime trail vs. water. *Euglandina rosea* significantly favored branches with slime trails vs. water, choosing the branch with slime trail 90% of the time ($P < 0.001$). Predatory snails exhibited no significant preference between *B. similaris* and *A. fulica* ($P = 0.820$), or *B. similaris* and *A. lila* ($P = 0.260$). However, *E. rosea* showed a significant preference for native *Achatinella lila* over invasive *Achatina fulica* ($P = 0.040$), the intended target species of the biological control program.

Key words: Hawaiian tree snails, predatory snails, *Achatinella lila*

Euglandina rosea (Férussac, 1821) is a carnivorous, terrestrial pulmonate snail native to the southeastern U.S.A. that preys exclusively on gastropod molluscs (reviewed in Barker and Efford 2004). *Euglandina rosea* was introduced to Hawaii and many other Pacific islands as a biological control method, targeting primarily the terrestrial agricultural pest species *Achatina fulica* Bowdich, 1822, the giant African snail (Mead 1979, Cowie 2001). Together with a number of other infamous, attempted bio-control programs in Hawaii that failed to control the intended pest species, this effort backfired catastrophically (Howarth 1991, Henneman and Memmott 2001, Holland *et al.* 2008), and has led directly to range reductions and extinctions of the native terrestrial island snail fauna (Clarke *et al.* 1984, Cowie 2001, Lydeard *et al.* 2004). *Euglandina rosea* was first released in Hawaii in 1955 by the Board of Agriculture and was subsequently introduced throughout the Pacific and Indian Oceans by officials in French Polynesia, Samoa, Mauritius, Micronesia, and elsewhere (Mead 1979, Griffiths *et al.* 1993, Civeyrel and Simberloff 1996, Cowie 2001). *Euglandina rosea* has since become a primary destructive agent of native Hawaiian and other island snails including arboreal species (Christensen 1984, Cowie 2001). The conservation and resource management community in the Hawaiian Islands has been intent on finding effective ways to attract, capture, and kill *E. rosea* for a number of years (Brenden Holland, pers. obs.). Although very little is presently known about the prey tracking and feeding behavior of this species in Hawaii, gaining understanding of these underlying mechanisms and strategies will provide important information that ultimately should be applicable to control efforts.

As in many animal groups, gastropods track and locate mates and food resources by detecting and following chemical trails (Chase 1986, Clifford *et al.* 2003, Shaheen *et al.* 2005). Trail tracking behavior is known from both predatory and non-predatory snails. Predatory snails have been shown to follow mucous trails in terrestrial, marine, and aquatic ecosystems (Chase 1986, Levri 1998, Davis 2005). At the present time, although an important topic, the underlying physiological and biochemical basis for prey mucous trail recognition by *E. rosea* is poorly understood and warrants further investigation.

Euglandina rosea feeds on slugs and other snails by tracking their prey through detection of small, water-soluble components in prey slime using specialized elongated lips (Clifford *et al.* 2003). Although most commonly encountered on the ground in Hawaii, *E. rosea* is known to climb trees in pursuit of prey (Davis *et al.* 2004), including of course, tree snails (Hadfield *et al.* 1993). Therefore, as in experiments focusing on tracking behavior in snail-eating flatworms (Sugiura and Yamaura 2009), studies of trail tracking behavior of *E. rosea* were conducted on tree branches. However, *E. rosea* behavior has previously not been observed on such materials, but has been studied on polyethylene sheets or glass plates (Cook 1985, Clifford *et al.* 2003, Shaheen *et al.* 2005). In addition to *Achatina fulica* and *Achatinella* spp., *E. rosea* have also been observed to prey on the common introduced snail *Bradybaena similaris* (Rang, 1831) (Davis and Butler 1964). The Oahu tree snail *Achatinella lila* Pilsbry, 1914 was included in the trials as a representative of the endemic Hawaiian malacofauna.

Laboratory prey trail preference choice trials were conducted on y-shaped ohia lehua (*Metrosideros polymorpha*) tree branches to simulate arboreal conditions where the native tree snails live and where *Euglandina rosea* tracks and hunts native tree snails. Y-shaped branches stripped of leaves were suspended 15 cm off the lab bench to simulate the arboreal environment. Snail trails were carefully placed from the beginning of the y-junction to the tip of each branch without overlap (Fig. 1), yet at the y-junction the two trails were close enough that *E. rosea* could taste both trails simultaneously with their specialized sensory palps (Fig. 1). The sides (left or right of the y-junction) of the prey trails were randomized during each set of trials to avoid any directional bias that may have resulted from light sources or other visual or magnetic cues that could affect the snails' choice of direction. Branches were changed between trials. On the occasions when branches were reused, branches were vigorously scrubbed in scalding water and detergent, then rinsed and air dried, effectively removing all traces of slime trails. Fresh trails were produced prior to each pairwise trial. For each trial series, 20 *E. rosea* individuals were used (Table 1); each predatory snail was used only once per day.

Ten tree branches were used. Tree branch dimensions were as follows: mean length of straight element, labeled A, was 8.75 cm (*SE* 3.06), mean length of split elements after y-junction was 9.79 cm (*SE* 2.31) the average angle between branches B and C was 40° (Fig. 1). Foliage was removed before trials began, and snail mucous trails were removed between trials, or new branches were used. Dimensions of y-junction of branches used was such that as *Euglandina rosea* moved along the straight portion to the junction, it was allowed to choose which direction, either B or C (Fig. 1), to pursue. Point A shows the starting point for predatory snails, arrows show direction of travel. Within each of the four pairwise trials, B and C were randomized, to account for any inherent left-right bias in *E. rosea*. Prey choice experiments in pairwise trials were conducted with pairings of *Achatina fulica* vs. *Achatinella lila*, *A. lila* vs. *Bradybaena similaris*, *B. similaris* vs. *A. fulica*, and *A. fulica* vs. water.

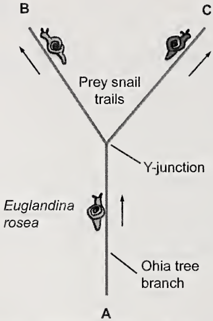

Figure 1. Schematic of experimental set-up, showing y-junction and relative dimensions of ohia lehua branch components. Laboratory prey trail preference trials were conducted on y-shaped ohia (*Metrosideros polymorpha*) tree branches.

All *Euglandina rosea* specimens were collected from the same locations in the Waianae Mountain range in Hawaii on the island of Oahu. Individual weight and shell length was recorded upon arrival to the lab. *Euglandina rosea* specimens ($N = 52$) were kept in plastic terraria in the laboratory (22 °C) and were fed *Bradybaena similaris* collected from various locations around the University of Hawaii (UH) at Manoa campus approx. once a week (Table 1). Previous studies indicate that trail following by *E. rosea* was not influenced by hunger (Clifford *et al.* 2003), but regular feedings were conducted to maintain the health of the specimens. The common *Achatina fulica* ($N = 5$) were also obtained on the UH campus. *Achatinella lila* ($N = 5$) occurs in the central Koolau Range on Oahu, and were kept in environmental chambers in the UH Tree Snail Conservation Lab. *Achatinella lila* was kept under permit by the USFWS and no individuals were harmed during these trials.

Since prey were not consumed by *Euglandina rosea* in these trials, one important consideration in our experimental design was the possibility that, since *Achatina fulica* is substantially larger than the other two experimental prey

Table 1. Total numbers of predator and prey snail specimens used. Shell length, width, and weight given are mean values with standard errors in parentheses.

Species	*N*	Shell length (cm)	Shell width (cm)	Weight (g)
Achatina fulica	5	53.36 (14.59)	28.8 (7.41)	21.03 (12.3)
Bradybaena similaris	10	8.35 (1.37)	4.66 (0.73)	0.16 (0.10)
Achatinella lila	5	15.83 (3.75)	7.76 (0.41)	0.76 (0.16)
Euglandina rosea	52	34.92 (9.54)	15.83 (3.75)	2.84 (2.07)

species, its slime trail could be wider, potentially causing confounding factors by the amount of trail material, rather than the intended variable. However, the tree branches used were standardized by their width to approx. 5–6 mm in diameter (within the width range of labial palps), so that branches were narrower than prey foot width, limiting prey size as a factor in the preference experiments.

To place slime trails on branches, *Achatina fulica*, *Achatinella lila*, and *Bradybaena similaris* were placed on the branch in an active state with foot protruding and were allowed to crawl along the length of the branch, with occasional directional prodding if the snail was inactive. Because of *A. fulica*'s large size relative to the tree branch, individuals occasionally required manual assistance to remain upright as they crawled along the length of the branch. Each trial was considered complete once *Euglandina rosea* made a choice and crawled to the tip of the branch furthest from the y-junction (Fig. 1). In all cases, slime trails were applied to branches, then prey snails removed, and predators immediately placed at the y-junction for the choice trial. Efforts were made to present fresh mucous trails to predators, normally less than 15 minutes old.

Three trials with 20 *Euglandina rosea* each were conducted with two prey species presented one at a time. In addition, an experiment was performed using 20 *E. rosea* with *Achatina fulica* slime on one side of the branch and tap water on the other (Fig. 1). These trials were a control to ensure that *E. rosea* was following mucous rather than following the branch for an opportunity to hydrate. Tracking frequencies were analyzed using a two-tailed binomial test (Iwai *et al.* 2010).

The results of the control trial showed clearly that *Euglandina rosea* pursued the *Achatina fulica* path significantly more often (18 out of 20 replicates) than the water path, supporting that *E. rosea* was indeed attracted to slime rather than water (Table 2).

However, no significant preference between *Bradybaena similaris* and *Achatina fulica* was observed, with nine predatory snails selecting *A. fulica* and 11 choosing *B. similaris* out of 20 replicates (two-tailed binomial test: $P = 0.820$). In trials between *B. similaris* and *Achatinella lila*, *Euglandina rosea* chose *B. similaris* 13 times and *A. lila* 7 times out of 20 trials ($P = 0.260$), suggesting that *E. rosea* had no preference between *B. similaris* and *A. lila* (Table 1).

Finally, in trials of *Achatinella lila* vs. *Achatina fulica*, the native Hawaiian tree snail was chosen 15 times out of 20 ($P = 0.040$). While not strongly significant, the results show for the first time that *Euglandina rosea* preferred an endemic, endangered species rather than its intended biocontrol target.

Previously published laboratory choice and gut content studies of field-collected individuals indicated that adults of *Euglandina rosea* preferred snail species much smaller than themselves (Cook 1985, Griffiths *et al.* 1993, Meyer and Cowie 2010). Whether *E. rosea* can discriminate among prey snail species using mucous trails had until now remained unclear although *E. rosea* has been known to discriminate between conspecific and prey snail trails (Clifford *et al.* 2003). Our trail-choice experiments indicated that *E. rosea* can discriminate between *Achatinella lila* and *Achatina fulica* trails.

Interestingly, *Euglandina rosea* showed no preference between the Oahu native *Achatinella lila* and introduced *Bradybaena similaris.* Implications here suggested that in areas where both prey species are present, neither had any immediate survival advantage over the other. However, *B. similaris* develops, matures, and reproduces at a much faster rate than *Achatinella* spp., giving the introduced species an advantage over the live bearing and late maturing *Achatinella* spp. There are forest regions on Oahu where both species co-occur, such as Puu Hapapa, where the majority of the *E. rosea* used in this study were collected. The *E. rosea* used in this study may have fed on both species in the wild and were therefore locally adapted to feeding on these prey. This could be a confounding factor causing preference of locally available prey over *Achatina fulica* though no preference was exhibited

Table 2. Trail preference results showing the number of times each prey trail was chosen by Euglandina rosea. Each trial series consisted of 20 replicates, table includes P values from two-tailed binomial tests, where P values with asterisk (*) were statistically significant.

Trial series	Total number of times chosen	*P* value
1	*Achatina* (11) vs. *Bradybaena* (9)	0.820
2	*Achatinella* (7) vs. *Bradybaena* (13)	0.260
3	*Achatinella* (15) vs. *Achatina* (5)	0.040*
4	*Achatina* (18) vs. water (2)	<0.001*

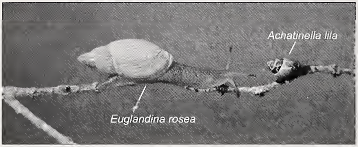

Figure 2. Adult *Euglandina rosea* tracking *Achatinella lila* that has just crawled along the branch leaving a fresh mucous trail. Prey snails were removed from the branch prior to placing *E. rosea* on the branch to avoid confounding olfactory or visual factors. Snail tracks were removed from bark between trials by scrubbing in scalding water and detergent, rinsing, and air drying.

for *B. similaris* vs. *A. fulica*. Future studies on prey preference in *E. rosea* might take prey ranges into account, and test prey collected from areas where *B. similaris* is present but species of *Achatinella* are absent, as well as areas where *Achatinella* spp. are present but *B. similaris* is absent, to evaluate the possible role of local adaptation in prey choice.

In the Hawaiian Islands, the geographic distribution of *Euglandina rosea* ranges from sea level to mountain peaks and ridges at about 1000 m, whereas *Achatina fulica* occurs in lower elevation habitats, likely allopatric with *Achatinella lila*. In addition *A. fulica* is a ground-dwelling species and has not been observed to climb ohia trees. Therefore it is highly unlikely that *E. rosea* would encounter a choice between native tree snails and African snails in the field.

Although resource managers in Hawaii have long speculated as to whether native island snails might be preferred to the intended biocontrol target species *Achatina fulica*, this is the first study to conclusively document this phenomenon. The data presented here provide the first concrete evidence that, when offered fresh mucous trails of the giant African snail and an endemic Oahu tree snail, there is a clear preference for the tree snail. In addition, in unpublished laboratory trials, we observed that even when *Euglandina rosea* attempted to attack larger *A. fulica* adults, it usually failed, due presumably to the large size disparity. Yet surprisingly, well-intentioned agriculture agency personnel from island nations where *A. fulica* is present periodically propose release of *E. rosea* as a solution. This study provides strong support against continued release of *E. rosea*, for any reason.

Knowledge of prey preferences of *Euglandina rosea* may ultimately help in the design and construction of attractant traps for *E. rosea* that could be used throughout the Pacific islands to control and ultimately exterminate *E. rosea* to preserve native and endemic fauna.

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Symposium on "Molluscan Biogeography: Perspectives from the Pacific Ocean" at the 76th Annual Meeting of the American Malacological Society, 2010

Peter B. Marko[1] **and Alan J. Kohn**[2]

[1]Department of Biological Sciences, Clemson University, Clemson, South Carolina 29634 U.S.A.
[2]Department of Biology, University of Washington, Seattle, Washington 98195, U.S.A.

Correspondence, Peter B. Marko: pmarko@clemson.edu

This issue of the *American Malacological Bulletin* includes four papers from the symposium on "Molluscan Biogeography: Perspectives From the Pacific Ocean," held at the joint annual meeting of the American Malacological Society and the Western Society of Malacologists in San Diego, California, on June 29th 2010. We focused the symposium on the Pacific region due to the wide range of macro-oceanographic environments: long linear coastlines, one- and two-dimensional archipelagoes, remote islands, and vast areas of open ocean. Past contrasts between the distributions and diversity of molluscan faunas across these different types of habitats have provided numerous important insights into the factors that influence species ranges and patterns of species diversity (*e.g.*, Taylor 1971, Valentine and Jablonski 1983, Perron and Kohn 1985, Vermeij 1987).

The symposium included presentations on species discrimination, speciation, life history evolution, island biogeography, and phylogeography; the papers published in this volume provide a broad representation of recent research in these subdisciplines. Alan Kohn analyzed life history and geographic range data with comparative methods to re-evaluate the relationship between egg size, larval developmental mode, and geographic range in the gastropod genus *Conus*. Phylogenetically-controlled comparisons provided strong support for the hypothesis that species with smaller eggs (and longer planktonic developmental times) do in fact have larger geographic ranges, an interesting biogeographic relationship all on its own, but also a fundamental component in hypotheses about the relationships among larval mode of development, geographic range, and species longevity in the sea (Jablonski and Lutz 1983, Jablonski and Hunt 2006). In contrast, a second contribution by Tom Duda, Marielle Terbio, Gang Chen, Semoya Phillips, Amy M. Olenzek, Dan Chang, and David W. Morris synthesized phylogeographic patterns across the tropical Pacific among four species of *Conus*, emphasizing the importance of dispersal capability and recent gene flow as an explanation for an absence of obvious phylogeographic concordance among four widespread tropical Pacific species. Despite limited genetic differentiation across much of each of these species' ranges (presumably as a consequence of planktonic dispersal), the data also suggest that rare jump dispersal events and peripheral isolation have resulted in considerable genetic structure in geographically distant populations of some but not all species, and may represent an important route to speciation in the tropical Pacific.

Suzanne Williams, Andie Hall, and Piotr Kuklinski used a combination of DNA sequence data and shell shape data to investigate the power of Elliptic Fourier analysis to discriminate among living and fossil species of the turbinid gastropod genus *Lunella* from the tropical western Pacific and Indian oceans. They reported that morphological divergence is greatest between sympatric taxa.

Christine Parent's conceptually-related paper evaluated the contribution of within-island speciation to patterns of diversity among three lineages of terrestrial snails: the Galápagos Bulimulidae, the Hawaiian Succineidae, and the Hawaiian Achatinellinae. Parent showed that within-island speciation has contributed substantially to patterns of species diversity in each archipelago but that the historical factors associated with speciation within islands appear to differ among the taxa, with either island size or habitat heterogeneity having the greatest influence over rates of speciation within islands. The surprisingly large amount of within-island speciation inferred for each of these lineages emphasizes the need for models that incorporate speciation into formal quantitative theories of island biogeography (Rosindell and Phillimore 2011).

Despite the variety of ideas, hypotheses, and questions that each of the published papers addressed, together they demonstrate the importance of studying biogeographic processes on different spatial and taxonomic scales. In *Conus*, Duda *et al.*'s conclusions about the stochastic nature of long distance dispersal that undoubtedly characterize the biogeographic histories of many species in the tropical Pacific complements the more deterministic relationship between dispersal potential and range size that has emerged from Kohn's work (e.g. Vermeij 1987, Palumbi *et al.* 1997, Lessios and Robertson 2006). Parent's focus on within-island diversification processes and Williams *et al.*'s (2011) suggestions about sympatric divergence together indicate that even though most molluscan speciation is likely initiated in allopatry, the small-scale habitat heterogeneity and transient geographic isolation that initiates divergence probably results in many opportunities for ecological interactions between recently split species, which may cause strong divergent selection on morphological, ecological, and behavioral traits (e.g. Marko 2005, Parent and Crespi 2009, Pfennig and Pfennig 2010, Rundell 2011, Krug 2011). If this is how speciation often proceeds in marine organisms (perhaps most often for linearly distributed nearshore species, see Valentine and Jablonski 1983), the implications for interpreting patterns of morphological change in the fossil record are potentially profound.

In addition to the published papers, other symposium participants presented work on other topics relevant to molluscan biogeography in the Pacific. David Jacobs discussed interoceanic transport in the trematode *Parorchis* and the gastropods *Cerithidea* and *Nutricola*, presumably accomplished by birds. Cynthia Trowbridge discussed biogeographic patterns of diversity among north Pacific saccoglossans, Doug Eernisse evaluated the significance of parapatric distributions towards understand patterns of diversification in eastern Pacific chitons, and Peter Marko reviewed the biogeographic and evolutionary responses of eastern Pacific molluscs to Pleistocene climate change.

The symposium highlighted the need for additional spatio-temporal perspectives on biogeographic processes, particularly those leading to speciation and diversification. Too few studies have combined fine-scale sampling of diverging populations and species with complementary studies of the fossil record. Patterns of sudden morphological change in the fossil record could represent peripatric speciation as envisioned by Eldredge and Gould (1972), but could also reflect other processes such as colonization of more distantly related species (Glaubrecht 2011), secondary contact and character displacement (Marko 2005), and taxon cycles (Ricklefs and Bermingham 2002). Studying extinction rates over evolutionary timescales is impossible without examination of the fossil record (Quental and Marshall 2010, Rabosky 2010, Losos 2011), and the potential for extinction to complicate histories inferred strictly from neontological data is potentially high; incomplete taxon sampling of living species alone is a problem in many neontological studies (Marko and Jackson 2001, Alroy 2002, Agapow *et al.* 2004, Isaac and Purvis 2004, Moran 2004, Laurin 2010). This call for more integration between neontological and paleontological perspectives is easier said than done: the taxa with the very best fossil records are often those that live in habitats that are hard to access in the Recent (sediments and deep water). Nevertheless, malacologists are uniquely poised to address biogeographic questions from a wide range of perspectives.

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Egg size, life history, and tropical marine gastropod biogeography*

Alan J. Kohn

Department of Biology, University of Washington, Seattle, Washington 98195, U.S.A.
Correspondence, Alan J. Kohn: kohn@uw.edu

Abstract: Comparative studies of egg size and larval morphological characteristics coupled with estimates of dispersal abilities enabled Kohn and Perron (1994) to predict attributes of geographic distribution patterns of Indo-West Pacific *Conus* species that vary widely in developmental mode. However, because at that time, no species-level phylogenetic hypothesis had ever been proposed for *Conus*, we were constrained to treat each species as an independent entity. During the past decade, however, molecular phylogenetics of *Conus* has progressed to the point where character mapping and the method of phylogenetically independent contrasts can evaluate the importance of phylogenetic relatedness to biogeographic patterns. Analyses of species in two well-supported clades with data on ranges of trait variations suggest that significant evolutionary associations remain among developmental traits that affect dispersal ability after accounting for phylogenetic relationships. These are clades of molluscivorous and piscivorous *Conus* species, both exclusively in the Indo-Pacific region. As yet inadequate data on both development types and biogeography exist to determine whether phylogenetic relatedness predicts biogeographic patterns elsewhere in this hyperdiverse genus.

Key words: geographic range, larval development, *Conus*, phylogeny, independent contrasts

How the geographic distribution of invertebrates relates to their reproduction and larval development has been a contentious issue in marine biology since Gunnar Thorson posed the basic question more than sixty years ago (Thorson 1950). The adults of many benthic molluscs and other invertebrates move little or not at all, yet many species range remarkably broadly, for example from Hawaii and Tahiti across the Pacific and Indian Oceans to East Africa. A few species have even crossed the formidable East Pacific Barrier to colonize the west coast of tropical America (Duda and Lessios 2009). They achieve this despite weakly swimming planktonic larvae that are transported passively by ocean currents. The larvae either feed and grow or survive by metabolizing stored yolk, sometimes for weeks, months, or even years (Strathmann and Strathmann 2007). In these respects marine benthic invertebrates resemble land plants, whose small seeds may disperse over broad areas while the parents remain in place. Their life histories differ strikingly from phylogenetically more closely related land animals, particularly vertebrates, whose adults more often disperse and whose young are typically relatively large when born but altricial and less mobile than adults.

Also partly analogous to the seed plants on land, developmental modes among benthic invertebrates range from the broadcast spawning of millions of small, unprotected eggs into the sea to brooding or otherwise protecting far fewer but larger, developmentally advanced offspring. These young recruit directly as benthic juveniles into the parent population, but the planktonic larvae that develop from the small eggs of species with high fecundity may be transported over vast distances. These dramatic differences in fecundity and egg size frequently occur among congeneric species, and they lead to considerable interspecific variation in dispersal potential as a function of developmental mode. They may also profoundly impact later life history characteristics, species longevity, and speciation rates as well as patterns of geographic distribution.

Of course many factors other than dispersal ability also affect biogeography. They include regional and global environmental processes and the spatial distribution of particular resources that organisms require at different life stages. Deterministic hypotheses advanced to explain observed biogeographic patterns of species are typically based on *dispersal*: distribution depends on the organisms' intrinsic ability to spread, or *vicariance*: distribution depends on geologic and climatic events in the extrinsic environment that split formerly continuously distributed ancestral species into subsequently allopatric sister species.

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Rigorous tests of these hypotheses are difficult. They are not all mutually exclusive, their appropriate time scales differ widely, and they are difficult to refute because experimental studies at global or regional scales are not feasible. One must rely on descriptive-correlative approaches, and other major obstacles pertinent to benthic invertebrates impede understanding. The plankton continues to be a "black box;" larvae are released into it and recruit out of it, and many factors that are difficult to observe directly and to manipulate experimentally affect their lives. However, the hypothesis that dispersal ability strongly influences geographic distribution is refutable. If species with the longest pre-competent planktonic periods also have the broadest ranges, the dispersal hypothesis is supported. Evidence favoring the contrasting null hypothesis in species that have both long planktonic stages and narrow geographic ranges refutes the dispersal hypothesis. In such cases other factors such as vicariance and ecological determinants must influence geographic ranges more importantly than dispersal ability.

Gastropods are especially favorable subjects for such investigation, because their larval shells: 1) encode information on developmental mode (Thorson 1950, Shuto 1974), 2) are often retained throughout life as the apex of the adult shell, and 3) are often preserved in the fossil record, giving access to developmental information about ancient taxa (Jablonski and Lutz 1983, Jablonski and Hunt 2006, Hendricks 2009). The hyperdiverse, mainly circumtropical neogastropod genus *Conus* provides a particularly amenable system to test the dispersal hypothesis because its unusually large number of similar species offers sufficient material for statistical tests. Also, because Kohn and Perron (1994) had studied aspects of comparative development of Indo-Pacific *Conus*, they took advantage of these data sets to demonstrate that, as predicted by the dispersal hypothesis, length of precompetent planktonic period correlated with several measures of geographic range extent. However, concluding causation from correlation is always questionable, and like many other studies of comparative biology at that time, ours was constrained by having to treat each species as an independent entity. Although the method of phylogenetically independent contrasts and other comparative methods had been developed to address the role of phylogenetic history in trait evolution (Felsenstein 1985, Harvey and Pagel 1991, Garland *et al.* 1992), and Kohn and Perron (1994) appreciated that some *Conus* species were closely related while others were more distant cousins, no hypothesis of species-level phylogenetic relationships in *Conus* had ever been proposed; this precluded us from applying the available methodology.

The situation soon changed, however, and within five years the pioneering work of Thomas Duda began to produce species-level phylogenetic hypotheses based on mitochondrial and nuclear gene sequence data (Duda and Palumbi 1999a, 1999b, see also Monje *et al.* 1999). These studies have burgeoned in the last decade, and a mtDNA-based species-level phylogenetic hypothesis now incorporates nearly 40% of the 600 or more species in the genus (Meyer *et al.* unpubl. data). At present, two well supported clades within this phylogeny include enough species whose relevant traits are adequately known to use phylogenetically independent contrasts. This paper briefly reviews *Conus* development and life history and prior phylogeny-free analyses of the relationship between dispersal ability and geographic range extent of Indo-Pacific species, and then reports the first attempt to test the dispersal hypothesis more rigorously by assessing the role of non-independence due to phylogeny.

MATERIALS AND METHODS

The following section briefly reviews reproduction and early life history of *Conus* and prior studies of how dispersal ability relates to geographic ranges, mainly summarized from the more extensive account in Kohn and Perron (1994). We determined geographic distribution patterns of Indo-West Pacific *Conus* from published locality records and data from collections in major museums compiled for a taxonomic manual (Röckel *et al.* 1995) with areas determined from range maps by the criterion of minimum convex polygons (Sedgewick 1988), computed in arbitrary units. Ranges are represented as percentages of maximum range, taken as the area spanned by the most widely distributed Indo-Pacific species, *Conus ebraeus* (Kohn and Perron 1994, see also Duda and Lessios 2009). These data were arcsine-transformed for statistical analyses.

Kohn and Perron (1994) selected time of the precompetent period, designated Tᴘ, the minimum number of days from hatching to metamorphic competence, as the early life history attribute most relevant to biogeography with available data. Because it provides a proxy of time for spread of veliger larvae by ocean currents, we assumed this duration to objectively estimate dispersal potential (see also Strathmann 2007). It is based on direct observations in the laboratory and estimates from egg size described in the next section. As yet no method exists to assess planktonic durations more accurately. We used the regression of precompetent period on egg size derived from larvae of 11 Indo-West Pacific *Conus* species reared under identical laboratory conditions through settlement and metamorphosis as a model to estimate the unknown minimum precompetent periods of the 50 additional species for which we knew egg size only. We then tested the dispersal hypothesis of biogeography by determining the relationship of geographic range size to dispersal potential. Such relationships that ignore phylogeny are termed "species correlations" (Price 1997). Geographic range area is normalized by dividing by each species' range area by that of the most widely distributed species, *C. ebraeus*, to facilitate comparison of data on different scales. Dispersal potential is expressed as Tp, determined in the laboratory or estimated from egg size (Kohn and Perron 1994).

Because species-level phylogenetic hypotheses are now available for *Conus*, distinguishing interspecific variation associated with phylogeny from variation that is independent of common ancestry should be possible. Here the effect of phylogeny on the observed correlation and regression of these traits is assessed by the method of phylogenetically independent contrasts—"contrast correlations" in Price's (1997) terminology—initially proposed by Felsenstein (1985). This method corrects for phylogenetic non-independence, that is phenotypic associations between traits due to their inheritance from common ancestors earlier in the species' evolutionary history, as opposed to those that evolved following divergence from their last common ancestor. Avise (2006) summarizes the basics and Harvey and Pagel (1991) give details of this approach.

The phylogenetic reconstruction used in the Results section is derived from concatenated sequence comparisons of three mitochondrial genes (12S, 16S, and CO1) (Meyer *et al.* unpubl. data). These results expand but are generally congruent with the earlier published trees of Duda and others (1999a, 1999b, 2001, 2005). The phylograms were rendered with FigTree (http://tree.bio.ed.ac.uk/software/figtree/).

Phylogenetically independent contrasts of minimum precompetent period and geographic range area were calculated with COMPARE (Martins 2004). It assumes the null hypothesis that characters evolve independently and randomly by Brownian motion, and a molecular clock, with all nodes equidistant from the root (Felsenstein 1985, Martins 2004, Van de Peer 2009). COMPARE determines contrasts at all nodes in the tree, a total of *N*-1 where *N* = number of taxa. Terminal branch lengths varied considerably on the mtDNA tree, and independent contrasts were also calculated from a tree with branch lengths in Kimura 2-parameter distances, to compare with the results under assumptions of COMPARE. For this analysis branch lengths on the pruned trees were calculated as the sums of branch lengths on the 3-mt-gene tree (Meyer *et al.* unpubl. data).

In addition, contrasts were also examined with a more conservative test proposed by Felsenstein (1988, see also Ackerly 2000) that avoids assuming the Brownian motion null hypothesis of phenotypic evolution. It employs only those contrasts between species pairs with no common branches in the path between them, giving *N*/2 contrasts, or (*N*-1)/2 for a tree with an odd number of species. The proportion of contrasts with matched vs. mismatched signs of the change in the two traits is subjected to the non-parametric sign test.

Paired graphs for sets of species that are both members of well-supported clades in the current species-level molecular phylogeny and have available appropriate trait data, i.e., for both precompetent planktonic periods and geographic ranges, show the extent to which accounting for phylogeny changes our interpretation of the relationship between planktonic period and geographic range from the phylogeny-free approach used by Kohn and Perron (1994).

REPRODUCTION, DEVELOPMENT AND EARLY LIFE HISTORY OF *CONUS*

As in most prosobranch gastropods, the sexes are separate in *Conus*, fertilization is internal, and eggs are deposited in tough capsules typically affixed to the protected undersides of coral slabs or boulders. Up to 100 capsules, each containing from ten to 50,000 ova depending on the species, are deposited in each clutch or egg mass. Oviposition may take a day or more; females then leave the clutch. Egg size is species-specific and ranges from 125 μm to 1 mm, an unusually broad range for a single genus (Fig. 1). Egg size is

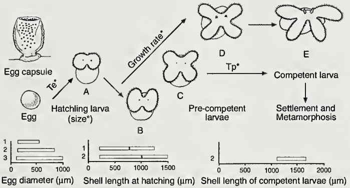

Figure 1. Capsule summary of the early life history of *Conus* species from oviposition of the fertilized egg within its protective capsule to metamorphosis. The regressions given in Table 1 enable prediction of the attributes indicated by * if egg size is known. Diagrams A–E represent the development of species with small (diameter ca. 125–250 μm). The diagrams also represent the hatching stage larvae of species with varying egg size, as follows: **A**, *C. lividus* Hwass (mean egg diameter 142 μm); **B**, *C. flavidus* Lamarck (193 μm); **C**, *C. striatus* Linnaeus (254 μm); **D**, *C. marmoreus* Linnaeus (417 μm); **E**, *C. pennaceus* Born (487 μm). $\mathbf{T_E}$, duration of embryonic or prehatching development; $\mathbf{T_P}$, duration of precompetent planktonic period. Bar graphs at bottom indicate known size ranges. Vertical lines on hatchling shell length bars indicate maximum size for species with planktonic larvae. **1**, species reared through metamorphosis in the laboratory; **2**, all known Indo-Pacific *Conus*; **3**, all marine prosobranch gastropods. Note the much smaller size range at competence than at hatching. Data from Kohn and Perron (1994).

strongly and inversely related to the number of eggs laid, both per capsule and per egg mass. Larger females lay more eggs than smaller conspecifics, but egg size appears to be a species-specific character that varies little with female size (Kohn and Perron 1994).

After oviposition, embryos develop within the capsules, apparently unaided by a supplementary energy source derived from either non-developing nurse eggs or nutritive intracapsular fluid as in some other gastropods (Perron 1981a, Jablonski and Lutz 1983). At hatching (Fig. 1A) the veliger larvae of many *Conus* species emerge and swim from the egg capsules to begin a planktonic period of obligatory feeding by velar cilia and growth (Fig. 1B-E). In other species, larvae emerge from the egg capsules at a somewhat later developmental stage, the veliconcha, immediately capable of benthic existence (e.g. Kohn 1993).

Whether or not a planktonic stage is present, larval development and growth in *Conus* follow a characteristic trajectory, manifested most strikingly by the velum, the larva's swimming and feeding organ. Appearing first as a small, bilobed ciliated structure, the velum slowly enlarges and the lobes bifurcate and elongate (Figs. 1A–E). In species with egg diameter larger than 470 μm, the entire course of velum development takes place within the egg capsule, and the velum often elongates less than shown in Fig. 1E (Kohn 1993). In species with the smallest eggs, hatching occurs sooner (Fig. 2), when the velum resembles that shown in Fig. 1A, and the remaining stages (Figs. 1B–E) develop while the planktonic larva is swimming and feeding. Larvae developing from intermediate-sized eggs hatch at intermediate stages of velum development. The ontogeny of the velum in species with small eggs thus consists of a developmental series resembling the hatchlings of species with progressively larger eggs (Kohn and Perron 1994).

Striking interspecific variability in egg size, fecundity, hatching type and minimum length of time spent in the plankton before metamorphosing and entering the adult habitat characterizes the genus *Conus*. To understand how these attributes are interrelated, we reared 13 Indo-West Pacific species through metamorphosis in the laboratory, 11 of them under identical conditions (Perron 1980, 1981a, 1981b, Kohn and Perron 1994). Figure 2 summarizes their embryonic period (T_E), the number of days between oviposition and hatching, and the precompetent planktonic period (T_P). $T_E + T_P$, indicated by the total height of the bars, equals the total development time from oviposition to metamorphic competence. Hatching size is maximum dimension of the larval shell, measured along the axis of coiling, at the time of emergence from the egg capsule, and settling size is maximum shell dimension at metamorphosis (Fig. 1).

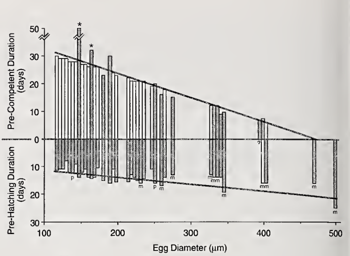

Figure 2. Relationships of durations of embryonic (prehatching) development (T_E) and of precompetent planktonic period (T_P) with egg size in species of Indo-Pacific *Conus* for which Kohn and Perron (1994) observed either or both durations. Each bar represents minimum total development time from oviposition to metamorphic competence, partitioned into T_E (below 0 line) and T_P (above 0 line). Stippled bars represent observed values and open bars, values estimated from regression equations in Table 1. The T_P regression line is based on data for 11 species (indicated by stippled bars) reared through metamorphosis under identical conditions; culture conditions differed for the two species indicated by asterisks above the bars. Divergence of the two regression lines to the left indicates that species with smaller eggs have longer total development time from oviposition to competence. Letters below bars indicate species in the molluscivorous (**m**) and piscivorous (**p**) clades with data used in phylogenetically independent contrasts. Modified from Kohn and Perron (1994).

Importance of egg size

A striking pattern in the regressions of embryonic and larval developmental characteristics across *Conus* species (Table 1) is that egg size explains a substantial portion of the variance in several other attributes of early life history. For three of these, size at hatching, length of precompetent planktonic period, and size of the first shell whorl, additional factors could not significantly improve the predictions based on the regressions on egg diameter.

Duration of embryonic development. The observed direct relationship of prehatching time with egg size (Table 1, Fig. 2) reflects the well-known inverse relationship between size and metabolic rate per unit of size (e.g. Steele 1977).

Size of larva at hatching. Basic physical and biological principles also predict the observed positive relation between egg size and size at hatching, and similar relationships are well documented in numerous gastropods (Lima and Lutz 1990) as well as other animal groups. In Indo-Pacific *Conus*, hatchling shell length ranges from about 240 μm in species

Table 1. Development and early life history characteristics of Indo-Pacific *Conus* species, showing the relationship of other parameters with egg size as the independent variable. The first two data rows show number of eggs as a function of egg diameter. The next seven rows show early life history characteristics as functions of egg diameter. All regressions are ordinary least squares. Data from Kohn and Perron (1994: Figs. 4.6–4.10).

Dependent Variable	No. of Species	Regression	R^2	F	P
Number of eggs per egg capsule	62	$Y = 1.6 \times 10^{10}X^{-3.0}$	0.72	156	0.0001
Number of eggs per egg mass	47	$Y = 1.1 \times 10^{11}X^{-2.7}$	0.63	75	0.0001
Prehatching period (d)	37	$Y = 0.02X + 8.9$	0.46	30	<0.001
Size at hatching (µm)	54	$Y = 2.49X + 163$	0.93	696	0.0001
Growth rate (µm/d)	6	$Y = 0.29X - 7.5$	0.59	5.9	0.07
Precompetent period (d)	11[1]	$Y = -0.09X + 40.8$	0.92	101	0.0001
Total development time (d)	37[2]	$Y = -0.07X + 51.7$	0.64	63	0.0001
Size at settlement (µm)	20	$Y = 0.001X - 1.7$	0.09	1.7	0.20
Diameter of first whorl (µm)	16	$Y = 0.87X - 53.7$	0.94	227	0.0001

[1] Species reared through metamorphosis in laboratory under identical conditions.

[2] Includes species with precompetent period estimated from egg diameter.

d Days

with the smallest eggs (125 µm) to more than 1200 µm in those with egg diameters ≥470 µm.

Growth rate in the plankton. The size of the egg and the developmental state of the larva at hatching strongly affect its growth rate in the plankton (Perron 1981b). These observed positive relationships, also general among marine invertebrate larvae (Marshall and Keough 2006), are likely attributable to the size and complexity of the velum at hatching and its subsequent growth pattern mentioned above and shown in Fig. 1. The size and other attributes of the peripheral ciliated food-collecting apparatus importantly affect larval feeding rates and the kinds of particulate food collected (Strathmann and Leise 1979, Hart and Strathmann 1994, Hart 1995). Larvae that hatch at larger sizes at more advanced stages of velum development thus likely begin planktonic life with more efficient means of obtaining food.

Duration of precompetent planktonic period. For the 13 *Conus* species reared through metamorphosis in the laboratory, this ranged 0-50 days and is very tightly correlated with egg size (Fig. 2, Table 1: $R = 0.92$). In addition to egg size, it could also be affected by growth rate in the plankton and the absolute amount of growth that must take place before metamorphic competence is attained. Duration of the precompetent period is particularly important because it is an indicator or proxy of larval dispersal capability.

Duration of total development to metamorphosis. Conus species with larger eggs have longer prehatching durations, but because their precompetent duration decreases more steeply, total development time from oviposition to metamorphosis (total length of the bars in Fig. 2) decreases with increasing egg size. In species with non-planktonic development, however, precompetent duration = 0 and time to hatching continues to increase with egg size beyond 470µm, resulting in greater total development time.

Size at metamorphosis. A striking feature of the data in Table 1 is that size at settlement, taken as the maximum dimension of the shell of the newly metamorphosed juvenile upon recruitment into the benthic environment, is not significantly correlated with any of the other larval developmental characteristics in the analysis. While size at hatching of planktonic veligers ranges over a factor of more than four (200–880µm), size at settlement of both planktonic and non-planktonic developers varies only by a factor of about 0.5 (1,120–1,650µm) (Fig. 1). The data suggest that *Conus* larvae must attain a minimum critical size prior to settlement and metamorphosis. According to this hypothesis, species with small eggs that hatch as small larvae after a short incubation period feed and grow more slowly but must grow more in the plankton than larvae with larger hatching sizes. The requirement of a longer precompetent planktonic period to attain critical size is thus likely to increase passive dispersal. Those that hatch as larger larvae, from larger eggs and after a longer incubation period, can grow more rapidly and attain competence to metamorphosis sooner, and hence are likely to settle at more philopatric locations. These relationships suggest that two sets of selective pressures determine larval life history patterns in *Conus*, the first controlling egg size and the second, size at settlement into the adult habitat.

Pre- and post-hatching mortality rates, and the absolute amount of energy that mothers are able to allocate to reproduction overall and per offspring, likely strongly influence evolution of egg size. In contrast, the food requirements of newly metamorphosed juveniles and the nature of predators and other challenges encountered in the adult habitat are likely important determinants of settling size (Nybakken and Perron 1988, Wray 1995, Strathmann 2007).

Correlation and causality. The relationships described above and summarized in Table 1 indicate that egg size is a key variable that may control pre-hatch duration, size at hatching, larval growth rate and extent of growth in the plankton, and the minimum duration of the precompetent period. Given egg size, these regressions allow prediction with considerable confidence of early life history characteristics in other *Conus* species for which we know egg size but lack information on other aspects of development. However, all of the data are descriptive-correlative, and the conclusion that egg size actually determines the other attributes associated with it is consistent with the hypothesis but does not conclusively demonstrate it. We do not know which life history trait is subject to the strongest selection pressure. For example, the correlation between egg size and hatching could have evolved as a result of selection for specific hatching sizes rather than for egg size.

"Allometric engineering"—experimentally altering egg size—has generated strong independent evidence for the primacy of egg size. Although not feasible in animals with determinate development such as *Conus*, studies on vertebrates and invertebrates with regulative development have demonstrated effects on life history parameters similar to those observed among *Conus* species that differ in egg size. Sinervo *et al.* (1992) manipulated yolk content to both reduce and increase egg size in lizards and showed that incubation time, size at hatching, and other attributes likely related to fitness vary with egg size in the same directions as observed in *Conus.* Reducing the size of echinoderm embryos by excision of blastomeres resulted in smaller hatching larvae that also grew more slowly than normal. Their size at metamorphosis depended on larval feeding regimen but not on egg size (Sinervo and McEdward 1988, Hart 1995). These studies strongly support the hypothesis that egg size is a cause rather than merely a correlate of the other observed differences.

Geographic distribution, egg size, and dispersal potential

Extending over 60° of latitude and across half the circumference of the globe, the Indo-West Pacific marine biogeographic realm covers more than a quarter of the earth's total ocean area. Many shallow-water benthic marine species associated with this region have long been considered to have extremely broad geographic ranges (Kohn 1983) and recent work has begun to provide molecular genetic verification (Duda and Lessios 2009). Superimposed on this general trend are complicating patterns of attenuation, endemicity and patchiness that have generated considerable controversy about causal factors in modern and historical biogeography (Jablonski and Hunt 2006, Paulay and Meyer 2006).

Kohn and Perron (1994) examined how accurately the dispersal hypothesis predicted three aspects of Indo-Pacific *Conus* distribution patterns. First, they predicted that species with broad, two-dimensional distributions encompassing isolated oceanic islands and archipelagoes would have smaller eggs and thus longer precompetent periods than those with predominantly linear or one-dimensional ranges along continuous continental coastlines, and nonplanktonic development should be more prevalent in the latter than in the former. In the Indo-Pacific region, 28% of *Conus* species on continental shores (Philippines, Indonesia and Thailand, and India to the Persian Gulf) had nonplanktonic development (10/36), in contrast to only 2% (1/41) in the oceanic area (Hawaii, Marshall Islands, Caroline Islands, and Indian Ocean islands), significantly different by the *G*-test for independence ($G = 6.05$; $P < 0.025$).

Second, boundaries of the earth's lithospheric plates far below the oceans were predicted not to impede the distribution of shallow water species with small eggs and good dispersal ability, and only species with nonplanktonic development were predicted to be confined to single plates. The vicariance hypothesis predicts greater importance of plate boundaries in determining species ranges, and supporting evidence has been reported for some Indo-Pacific fishes and gastropods (Springer 1982, Kay 1990). Two very diverse *Conus* faunas, in the Philippines on the eastern margin of the Eurasian Plate, and New Caledonia on the Indo-Australian plate, have about 100 and 85 shallow-water (< 20m) species, with 53% and 60% whose mode of larval development is known, respectively. More than 90% of these either 1) have a planktonic larval stage and occur over 4 or 5 lithospheric plates, or 2) have nonplanktonic development and are confined to the continental islands of a single plate.

The third and perhaps most direct test is whether the absolute area of the geographic range occupied by species varies inversely with their egg sizes and thus directly with the length of their precompetent periods. We tested this expression of the dispersal hypothesis by regressing absolute range area on observed and estimated precompetent planktonic periods. The regression (Fig. 3) accounts for 39% of the variance in absolute range for the entire data set of 61 species. This statistically highly significant result ($P = 0.0001$) indicates that the association of broader geographic range with longer precompetent planktonic periods is unlikely due to chance, but the data are not without exceptions. For example, the lower right outlier points in Fig. 3 represent the few narrowly distributed species with long precompetent planktonic periods, indicating that other, unknown factors may strongly influence distribution patterns. In addition, the dichotomy between species with planktonic and nonplanktonic development strongly influences the regression. Removing the latter and regressing range area on minimum

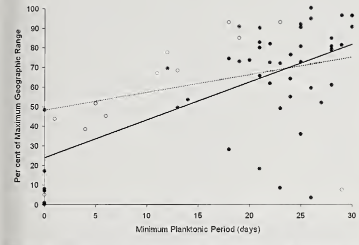

Figure 3. Relationship of geographic range area to observed (open symbols) or estimated (solid symbols) minimum precompetent planktonic period in Indo-Pacific *Conus*. Ranges are normalized to that of the species with the largest range, *C. ebraeus* Linnaeus. Ordinary Least Squares (OLS) regression (solid line): Y = 0.02X + 0.24; $R^2 = 0.39$; $F = 37.5$; $P = 0.0001$; $N = 61$. The dotted line is the regression for species with planktonic development only: Y = 0.01X + 0.489; $R^2 = 0.06$; $F = 3.3$; $P = 0.075$; $N = 52$. Modified and updated from Kohn and Perron (1994).

planktonic period of only those species whose larvae are able to swim at hatching (N = 52) reduces the significance of the regression ($P = 0.075$) but the trend remains evident. Lack of information at that time precluded the possibility of accounting for phylogenetic relatedness of species in the analyses.

RESULTS

During the past decade, species-level molecular phylogenetic studies in *Conus* have identified a number of well-supported clades within the genus (Duda *et al.* 2001, 2005, Espino *et al.* 2008, Meyer *et al.*, unpubl. data). However, relevant trait values with sufficient ranges for meaningful contrasts, specifically dispersal ability and range size, are known for enough species in only two clades to test whether our 1994 hypothesis relating these traits maintains support, or whether phylogenetic history of lineages constrains the observed relationships of developmental traits and biogeography.

These two clades differ markedly in feeding ecology, topology, and in the amount of pruning required to select taxa with available appropriate trait data for analysis. The "molluscivorous clade," comprising species that are specialized predators on other gastropods, probably originated only once in *Conus*; all known molluscivorous species are each other's closest relatives (Duda *et al.* 2001: Fig. 3, clade M1; Espino *et al.* 2008). Tree topology of the "piscivorous clade," comprising specialized predators on fishes, differs markedly, because piscivory likely originated more than once in the evolutionary history of the genus (Duda *et al.* 2001: Fig. 3, clades F1, F2, F3), and the heavier pruning required to accommodate an adequate sample size for analysis necessitated reducing these to a more artificial single tree.

Molluscivorous clade

This clade presently includes about 25 species (Meyer *et al.*, unpubl. data). The 12 shown in Fig. 4 occupy well supported positions in the phylogeny and their developmental and geographic range trait data are known. Figs. 5 and 6 compare the phylogeny-free species regression in this clade with the phylogenetically independent contrasts of dispersal ability and range area. The species regression of proportion of maximum geographic range occupied on minimum plankton period (Fig. 5) explains 75% of the variance and is highly significant ($P < 0.001$), as is the regression including only the 10 species with planktonic development ($P = 0.01$). Arcsin transformation of proportions of maximum range area (not shown) gave the same results.

The plot of phylogenetically independent contrasts in minimum planktonic duration and proportion of maximum range between species pairs (Fig. 6) shows that a significant regression ($P = 0.002$) remains in the molluscivore clade after excluding phylogenetic effects. Substituting observed branch lengths on the phylogram from K2P distances gave a similar regression of independent contrasts (Y = 1.57X; $R^2 = 0.66$;

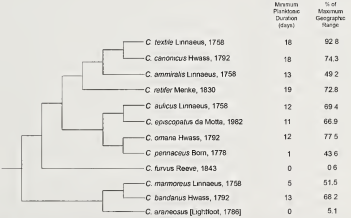
Minimum Planktonic Duration (days)
% of Maximum Geographic Range
C. textile Linnaeus, 1758 18 92.8
C. canonicus Hwass, 1792 18 74.3
C. ammiralis Linnaeus, 1758 13 49.2
C. retifer Menke, 1830 19 72.8
C. aulicus Linnaeus, 1758 12 69.4
C. episcopatus da Motta, 1982 11 66.9
C. omana Hwass, 1792 12 77.5
C. pennaceus Born, 1778 1 43.6
C. furvus Reeve, 1843 0 0.6
C. marmoreus Linnaeus, 1758 5 51.5
C. bandanus Hwass, 1792 13 68.2
C. araneosus [Lightfoot, 1786] 0 5.1

Figure 4. Pruned phylogram of the 12 molluscivorous clade *Conus* species with known precompetent period and geographic range trait values at right (from Kohn and Perron 1994) used to calculate independent contrasts. The phylogeny is derived from molecular sequence comparisons of 12S, 16S and CO1 mitochondrial genes, after Duda *et al.* (2001) with updates from Espino *et al.* (2008) and Meyer *et al.* unpubl. data.

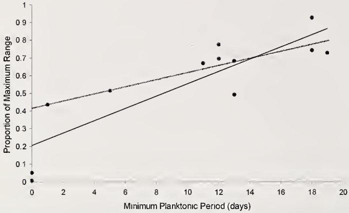

Figure 5. Relationship of geographic range area to minimum precompetent planktonic period in the clade of molluscivorous Indo-Pacific *Conus* species. Ranges are normalized to that of *C. ebraeus* Linnaeus, 1758, the species with the largest range. Solid line (all species): Y = 0.03X + 0.21; $R^2 = 0.75$; $F = 30$; $P < 0.001$; $N = 12$. Dotted line (species with planktonic larvae only): Y = 0.02X + 0.42; $R^2 = 0.59$; $F = 11.4$; $P = 0.01$; $N = 10$. Data from Kohn and Perron (1994).

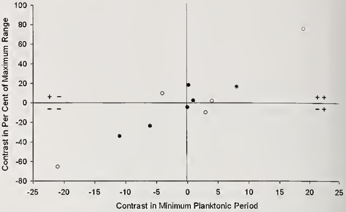

Figure 7. Bivariate scatterplot of phylogenetically independent contrasts in duration of precompetent planktonic period (T_p) and range area between species pairs without branches in common in the molluscivore (solid symbols) and piscivore clades (open symbols). Lines through origin highlight positions of contrasts for the sign test: matches (+ + and - -) in lower left and upper right quadrants; mismatches (+ - and - +) in upper left and lower right quadrants. Of the two evident ties in T_p among the 12 species (Fig. 4), *Conus furvus*-*C. araneosus* was dropped from the analysis and using the next decimal place resolved *C. canonicus*-*C. textile*. The other molluscivore clade contrasts are *C. ammiralis*-*C. retifer*, *C. aulicus*-*C. episcopatus*, *C. pennaceus*-*C. omaria*, and *C. bandanus*-*C. marmoreus*; data in Fig. 4. Thus for molluscivores, $N/2 = 5$; 0 mismatches; $P = 0.031$. The piscivore clade contrasts are *C. magus*-*C. consors*, *C. achatinus*-*C. catus*, *C. striolatus*-*C. stercusmuscarum*, *C. striatus*-*C. cinereus*, and *C. obscurus*-*C. geographus*; data in Fig. 7. For piscivores, $N/2 = 5$; 2 mismatches; $P = 0.5$. For all 10 contrasts in both clades: 2 mismatches; $P = 0.055$.

$F = 19.1$; $P = 0.001$), although this approach violates the Brownian motion null hypothesis of phenotypic evolution in COMPARE. All five independent contrasts between species pairs without branches in common, in the method that does not assume the Brownian motion model, matched in sign ($P = 0.031$) (Fig. 7). $N/2 = 5$ contrasts because the sign test does not accommodate ties—in this case two species with minimum planktonic period = 0. These results suggest that significant evolutionary diversification in developmental and biogeographic traits of sister taxa in the molluscivorous clade of *Conus* has occurred since divergence from their last common ancestors.

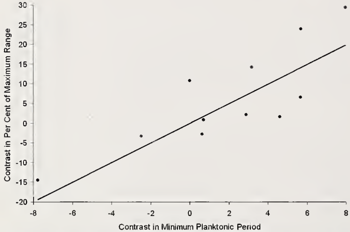

Figure 6. Bivariate scatterplot of standardized phylogenetically independent contrasts in range area and duration of precompetent planktonic period in Indo-Pacific *Conus* species in the molluscivorous clade in Fig. 4. Y = 2.5X; $R^2 = 0.64$; $F = 17.5$; $P < 0.002$; $N = 11$. Per cent of maximum geographic range values in Fig. 5 were arcsin transformed for this analysis. R^2 values are not comparable between Figs. 5 and 6, because the latter is a regression through the origin.

Piscivorous clade

Duda *et al.* (2001) identified three separate clades, identified as F1, F2, and F3, comprising 6, 3, and 3 Indo-Pacific fish-eating species, respectively. Subsequently we recognized a fourth clade comprising one species each from the Atlantic and Eastern Pacific regions as well as additional species in all three Indo-Pacific clades (Duda and Kohn 2005). Clade F3 is now the largest of these, with 16 species, Clade F1 has 13, and Clade F3, four, all Indo-Pacific (Meyer, *et al.*, unpubl. data). Appropriate trait data for determining phylogenetically independent contrasts were available for only ten of these, seven of them in clade F1 (Fig. 8).

The phylogeny-free species regression (Fig. 9) shows that range area is significantly correlated with minimum planktonic area for the ten species ($P = 0.04$). Arcsin

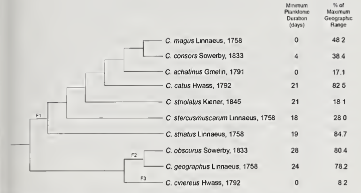
Minimum Planktonic Duration (days)
% of Maximum Geographic Range
C. magus Linnaeus, 1758 0 48.2
C. consors Sowerby, 1833 4 38.4
C. achatinus Gmelin, 1791 0 17.1
C. catus Hwass, 1792 21 82.5
C. striolatus Kiener, 1845 21 18.1
C. stercusmuscarum Linnaeus, 1758 18 28.0
C. striatus Linnaeus, 1758 19 84.7
C. obscurus Sowerby, 1833 28 80.4
C. geographus Linnaeus, 1758 24 78.2
C. cinereus Hwass, 1792 0 8.2
F1
F2
F3

Figure 8. Pruned phylogram of the 10 piscivorous clade *Conus* species with known precompetent period and geographic range trait values at right (from Kohn and Perron 1994) used to calculate independent contrasts. Omitted taxa include vermivorous clades between some piscivorous clades. The phylogeny is derived from molecular sequence comparisons of 12S, 16S and CO1 mitochondrial genes, after Duda *et al.* (2001) and Duda and Kohn (2005) with updates from Meyer *et al.*, unpubl. data. **F1, F2,** and **F3** indicate members of clades of piscivorous species recognized by Duda *et al.* (2001).

transformation of proportions of maximum range area (not shown) gave essentially the same result ($P = 0.04$). Although the trend remains when only the seven members of Clade F1 are included, the statistical significance of the species relationship does not (Fig. 9).

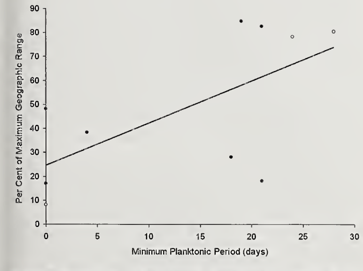

Figure 9. Relationship of geographic range area to minimum precompetent planktonic period in ten piscivorous Indo-Pacific *Conus* species. Ranges are normalized to *C. ebraeus,* the species with the largest range. $Y = 1.2X + 25.9$; $R^2 = 0.43$; $F = 5.9$; $P = 0.04$. For the seven members of Clade F1 only (solid symbols), $Y = 0.63X + 34.9$; $R^2 = 0.13$; $F = 0.7$; $P = 0.43$.

Application of the independent contrast method to all 10 piscivores also resulted in a significant regression of the contrasts for the two traits across species ($P = 0.02$; Fig. 10). As in the case of the phylogeny-free data, the regression of contrasts in minimum planktonic period and geographic range of only the seven Clade F1 members is not significant ($P = 0.21$). Substituting observed branch lengths from K2P distances on the piscivore phylogram gave a similar but less strongly supported regression of independent contrasts ($Y = 1.32X$; $R^2 = 0.51$; $F = 4.1$; $P = 0.08$), although as noted above this approach violates the Brownian motion assumption of COMPARE. Avoiding this assumption by applying the method of independent contrasts between species pairs without branches in common gave ($N/2 = 5$) contrasts, of which three pairs matched in sign ($P = 0.5$) (Fig. 7). Contrasts in both traits range much more widely in this clade, and those that differ less (closer to the origin in Fig. 7) depart farther from the general trend than in the molluscivore clade with its more uniform trend. The piscivorous species suitable for analysis represent more widely separated subclades on the phylogenetic tree of the genus (Duda *et al.* 2005, Fig. 7), but this cannot account for the entire distribution of the differences between the clades. Of the two species pairs that differed most in both traits, *Conus striatus-C. cinereus* (the extreme + + point in Fig. 10), are in different subclades, while *C. achatinus-C. catus* (the extreme – – point), are both in subclade F1.

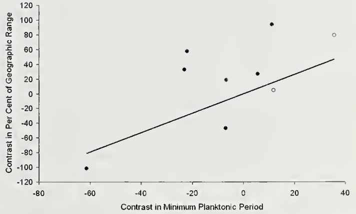

Figure 10. Bivariate scatterplot of standardized phylogenetically independent contrasts in range area and duration of precompetent planktonic period in ten Indo-Pacific piscivorous *Conus* species. $Y = 1.74X$; $R^2 = 0.57$; $P = 0.02$. Solid symbols represent contrasts in the Clade F1 taxa (Fig. 7). For these seven, the regression is not significant: $Y = 1.56X$; $R^2 = 0.29$; $F = 2.06$; $P = 0.21$. Per cent of maximum geographic range values in Fig. 9 were arcsin transformed for this analysis. R^2 values are not comparable between Figs. 9 and 10, because the latter is a regression through the origin.

Combining the results of all 10 contrasts from both molluscivorous and piscivorous *Conus* clades (excluding the tie; two are mismatches) in a single sign test is, although not conventionally quite statistically significant ($P = 0.055$), also consistent with the evolutionary association of developmental traits favoring dispersal and broad geographic ranges after accounting for phylogeny.

DISCUSSION AND CONCLUSIONS

Our prior, necessarily phylogeny-free analyses of 61 Indo-Pacific *Conus* species (Kohn and Perron 1994), summarized above and represented in Fig. 3, clearly supported the conclusions that egg size importantly affects dispersal ability and that the latter is an important determinant of geographic distribution patterns. Species with small eggs that hatch as early veliger larvae and have long precompetent planktonic periods in their life cycles and therefore greater dispersal potential also have significantly larger geographic ranges. However, these analyses considered the reproductive, life history, and biogeographic traits of *Conus* species as independent data points. Absence of a phylogenetic hypothesis prevented us from addressing the possibility of nonindependence due to phylogeny. Considering phylogeny is important, because if similarities in dispersal ability and geographic range between closely related species are due to inheritance from a common ancestor, increasing the number of species sampled does not increase the amount of information or, in statistical terms, the number of degrees of freedom. That is why "phylogenetic correction will inevitably but correctly conclude in many cases that comparative evidence alone is insufficient for the evaluation of a hypothesis" (Harvey *et al.* 1995).

The present study thus asked, do these results remain valid in the light of new phylogenetic data, or were we victimized by pseudoreplication by failing to account for similarities due to phylogeny? Two clades within the species-level molecular phylogeny of *Conus* provided rather small but statistically adequate numbers of species and ranges of correlated trait values, and thus the opportunity to approach this problem.

Among both the ten evolutionarily more closely related molluscivorous *Conus* species and the ten more disparate piscivorous subclade members, accounting for phylogenetic effects on life history and geographic range traits by the method of independent contrasts indicates that significant evolutionary diversification has occurred since divergence from last common ancestors. The demonstrated correlations and regressions between these traits that we identified in our earlier, phylogeny-free analysis (Kohn and Perron 1994) are thus concluded not likely to be due solely to phylogenetic history prior to the branching events that led to extant species.

This evidence for evolutionary divergence in dispersal ability and geographic range independent of earlier phylogeny is consistent with evidence of rapid evolutionary change in other, likely independent traits in *Conus*. The most striking of these is the high rate of diversification of peptide conotoxins following gene duplication and likely differential selection both within and among species (Duda and Palumbi 1999a, 2004, Conticello *et al.* 2001).

The Indo-Pacific molluscivore and piscivore clades in *Conus* include species that vary widely in both egg size and geographic range, a requirement for this study. Variation in egg size imposes important diversity on subsequent early life history, most notably in the precompetent planktonic larval period and hence dispersal of larvae. However, the majority of *Conus* species are neither molluscivores nor piscivores but are predators on polychaete annelids, and that is the most likely plesiomorphic character state for the genus (Duda *et al.* 2001). Unfortunately, this large array of species is presently less suitable for exploring the role of phylogeny in the relationship between developmental and biogeographic traits. In most Indo-Pacific vermivorous species whose development is known or can be estimated from egg size, small eggs hatch as veligers with long precompetent periods, egg size and hence estimates of the durations of these periods vary between rather narrow limits, and evolutionary trends in the direction of large eggs and lecithotrophic development appear to be rare, in contrast to its more common occurrence in the molluscivorous and piscivorous clades. For example, in one well supported clade of 13 species, sometimes referred to as the subgenus *Virroconus* (Meyer *et al.*, unpubl. data), for which egg diameter is known in seven, its range is only 165–185μm, with observed and estimated minimum precompetent periods of 25–29 days. The cloud of data points in the right-hand portion of Fig. 3 shows that correlation between minimum planktonic period and geographic area occupied is generally absent among Indo-Pacific species with this narrow a range of egg size.

Further testing of the dispersal hypothesis must thus await knowledge of egg size and developmental mode in additional species and geographic regions. Moreover, as Duda et al. (2012) cogently point out elsewhere in this volume, differences in dispersal ability are not the only determinants of different phylogeographic patterns. Physiological and ecological factors associated with recruitment can also importantly affect geographic distribution and population genetic structure. As yet the roles of these factors remain obscure. The current rapid expansion of phylogenetic information and inference should guide future research efforts in this direction.

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Patterns of population structure and historical demography of *Conus* species in the tropical Pacific*

Thomas F. Duda, Jr.[1,2], Marielle Terbio[1], Gang Chen[1], Semoya Phillips[1], Amy M. Olenzek[1], Dan Chang[1], and David W. Morris[1]

[1] Museum of Zoology & Department of Ecology and Evolutionary Biology, University of Michigan, 1109 Geddes Avenue, Ann Arbor, Michigan 48109, U.S.A.

[2] Smithsonian Tropical Research Institute, Balboa, Ancón, Republic of Panamá

Correspondence, Thomas F. Duda, Jr.: tfduda@umich.edu

Abstract. We compared patterns of genetic population structure and historical demography of several tropical Pacific *Conus* species that have similar though not completely overlapping distributions. Although these species possess similar life histories with planktonic larval durations of at least three weeks and exhibit genetic homogeneity across broad expanses of their distributions, they show distinct patterns of genetic population structure that are largely associated with genetic differentiation of geographically isolated populations. While previous analyses of *C. ebraeus* (Linnaeus, 1758) and *C. miliaris* (Hwass in Bruguière, 1792) detected high levels of genetic differentiation among isolated locations in the eastern Pacific and at Easter Island for each species, respectively, *C. chaldaeus* (Röding, 1798) does not show robust evidence of population differentiation across the East Pacific Barrier, albeit the sample size from the eastern Pacific is small. In addition, unlike *C. chaldaeus* and *C. ebraeus*, *C. sanguinolentus* (Quoy and Gaimard, 1834) exhibits a strong genetic break at Hawaii. Analyses of mismatch distributions suggest recent population expansion of *C. chaldaeus* and *C. sanguinolentus* as well as the Hawaiian endemic species *C. abbreviatus* (Reeve, 1843) during the past one million years as was observed previously for tropical Pacific populations of *C. ebraeus* and *C. miliaris*. Taken together, these results show that although high dispersal rates appear to genetically homogenize broadly distributed species in the tropical Pacific, stochasticity in long-distance dispersal likely instigates genetic differentiation of geographically isolated and peripheral populations and results in discordant phylogeographic patterns among even closely related species. Thus, population divergence and speciation in the tropical Pacific likely occur among populations at isolated locations though gene flow tends to prevent differentiation at broad geographic scales in species with high potentials for dispersal.

Key words: Conidae, phylogeography, genetic differentiation, population expansion, cytochrome oxidase subunit I

Phylogeographic investigations can illuminate the factors responsible for current and historical distributional patterns of species and the origins and maintenance of earth's biotic diversity. Indeed, comparative phylogeography is a powerful approach for identifying processes related to the origins of contemporary patterns of genetic population structure among sets of species inhabiting the same biogeographic region and can reveal historical vicariant events that have similarly influenced these species (Avise 2000). For example, similar patterns of genetic subdivision among Atlantic Ocean and Gulf of Mexico populations of multiple phylogenetically disparate species suggest that populations in these regions were vicariantly separated in the past (Reeb and Avise 1990). In addition, concordant patterns of genetic differentiation among populations of tropical marine species in the Indian and Pacific Oceans support a previous vicariant separation of these populations associated with past low sea level stands (Benzie and Stoddart 1992, McMillan and Palumbi 1995, Lavery *et al.* 1996, Miya and Nishida 1997, Williams and Benzie 1997, 1998, Duke *et al.* 1998, Benzie 1999, Duda and Palumbi 1999, Lessios *et al.* 1999, 2001, Williams *et al.* 2002, Bay *et al.* 2004, Teske *et al.* 2005, Reid *et al.* 2006, Crandall *et al.* 2008).

Many works have explored patterns of population genetic structure of a variety of species in the tropical Pacific, from Winans' (1980) allozyme studies of the milkfish *Chanos chanos* (Forsskål, 1775) to Crandall and co-authors' (2008) recent and impressively sample-rich phylogeographic studies of two neritid gastropod species based on analyses of mitochondrial gene sequences. The main conclusions from these investigations are that (1) patterns of population genetic structure differ considerably among species in the tropical Pacific and (2) dispersal ability and oceanic current patterns are subtly or in some cases strongly related to the degree of genetic subdivision among samples from different locations

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(*e.g.*, McMillan *et al.* 1992, also Palumbi 1994). Nonetheless, differences in dispersal ability alone are usually insufficient to account for different phylogeographic patterns among species in this region (*e.g.*, Palumbi *et al.* 1997, Crandall *et al.* 2008). Factors such as stochasticity in dispersal events (*e.g.*, Palumbi *et al.* 1997, Lessios and Robertson 2006) and physiological or ecological phenomena associated with persistence and successful recruitment to particular locations (*e.g.*, Hilbish and Koehn 1985, Lee and Ó Foighil 2004, Marko 2004, Kelly and Palumbi 2010) also affect genetic population structures of marine taxa.

Conus chaldaeus (Röding, 1798) and *C. ebraeus* (Linneaus, 1758) are sister species (Duda and Kohn 2005) that show nearly identical distributions and are two of just three transpacific *Conus* species that occur in both the Indo-West Pacific and eastern Pacific (Röckel *et al.* 1995) (Figs. 1A, 1B). These species also have similar estimated minimum planktonic larval periods of 25–27 days (Kohn and Perron 1994). Based on phylogenetic reconstructions, *C. miliaris* (Hwass in Bruguière, 1792) shared a most recent common ancestor with *C. chaldaeus* and *C. ebraeus* approximately 10 million years ago (mya) (Duda and Kohn 2005). The range of *C. miliaris* overlaps largely with distributions of both *C. chaldaeus* and *C. ebraeus* though it is the only of these three species that is established at Easter Island [note: although *C. ebraeus* is reported to occur at Easter Island (Röckel *et al.* 1995), it is only rarely observed at this location and does not appear to be established (Duda, pers. obs.)] and in extreme northwestern parts of the Indian Ocean, including the Red Sea; unlike *C. chaldaeus* and *C. ebraeus*, it is absent from Hawaii and the Marquesas (Röckel *et al.* 1995) (Fig. 1C). *Conus miliaris* has an estimated minimum pelagic larval period of 23–27 days (Kohn and Perron 1994). Röckel *et al.* (1995) report that *C. sanguinolentus* (Quoy and Gaimard, 1834) occurs throughout much of Indo-West Pacific, is absent at Easter Island and purportedly does not occur in the central Indian Ocean and at Hawaii (Fig. 1D). Nonetheless, as described below and as discussed by Walls (1979) and Röckel *et al.* (1995), this species is likely confused with *C. lividus* (Hwass in Bruguière, 1792) which occurs in parts of the central Indian Ocean and within the Hawaiian Archipelago (Fig. 1D). *Conus lividus* has an estimated minimum pelagic larval period of at least four weeks (Kohn and Perron 1994), but the life history of *C. sanguinolentus* has not been described. In light of the possibility that descriptions of the life history of *C. lividus* may have actually considered *C. sanguinolentus*, we suspect that *C. sanguinolentus* also has a planktonic larval phase of at least four weeks. *Conus sanguinolentus* and *C. lividus* are closely related, but are not sister species (the eastern Pacific species *C. diadema* (Sowerby, 1834) is the sister species of *C. sanguinolentus*), and shared a most recent common ancestor with *C. chaldaeus*, *C. ebraeus* and *C. miliaris* about 25–30 mya (Duda and Kohn 2005). Based on the similar life histories and present distributions of these species, we predict that they exhibit similar phylogeographic patterns and demographic histories as those determined previously for *C. ebraeus* and *C. miliaris*.

Previous analyses of phylogeographic patterns and demographic histories of two widespread *Conus* species in the tropical Pacific, *C. ebraeus* and *C. miliaris*, revealed similar patterns of population differentiation at geographically isolated locations (eastern Pacific and Easter Island, respectively), genetic homogeneity across large parts of the distributions of these species, and recent population expansion (Duda and Lee 2009, Duda and Lessios 2009). To compare patterns of population genetic structure and demographic histories of additional members of the marine gastropod genus *Conus*, including a sister species of *C. ebraeus* that exhibits an almost identical distribution in the Pacific and Indian Oceans, we examined sequences of a region of the mitochondrial cytochrome oxidase c subunit I (*COI*) gene of individuals of three widespread species, *C. chaldaeus*, *C. lividus* and *C. sanguinolentus*, from multiple geographic locations, and a species that is restricted to the Hawaiian Archipelago, *C. abbreviatus* (Reeve, 1843) that has a similar life history as the other species presented above (*i.e.*, a minimum planktonic larval phase duration of 26–32 days) (Kohn and Perron 1994), to address the following questions. Do *Conus* species with similar distributions as well as similar life history modes and anticipated high levels of gene flow as *C. ebraeus* and *C. miliaris* lack genetic population structure throughout large parts of their ranges in the tropical Pacific? Does *C. chaldaeus* exhibit genetic subdivision of Indo-West Pacific and eastern Pacific populations as observed for its sister species, *C. ebraeus* (Duda and Lessios 2009)? We also augmented the sample size of *C. ebraeus* from the eastern Pacific and obtained mitochondrial gene sequences from these specimens to more rigorously examine the putative genetic disjunction between the Indo-West Pacific and eastern Pacific that Duda and Lessios (2009) determined based on a relatively small sample size ($N = 10$) from the eastern Pacific. Although *C. abbreviatus* is largely restricted to the Hawaiian Archipelago (Röckel *et al.* 1995), we utilized information from this species to compare demographic histories of species with different range sizes.

MATERIALS AND METHODS

Specimens

We obtained specimens of *Conus abbreviatus*, *C. chaldaeus*, *C. lividus* and *C. sanguinolentus* from the field or from museum collections that came from many locations in the Indo-West Pacific, including Reunion Island (*C. sanguinolentus*), Papua New Guinea (*C. lividus* and *C. sanguinolentus*),

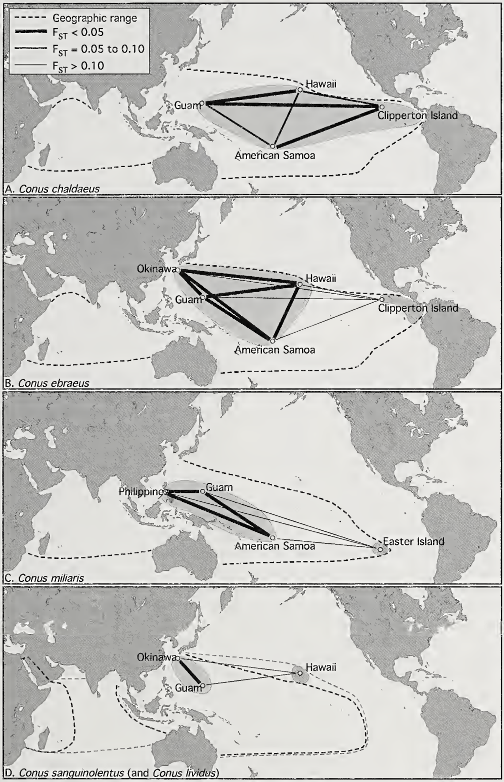
Geographic range
$F_{ST} < 0.05$
$F_{ST} = 0.05$ to 0.10
$F_{ST} > 0.10$
Hawaii
Guam
Clipperton Island
American Samoa
A. Conus chaldaeus
Okinawa
Hawaii
Guam
Clipperton Island
American Samoa
B. Conus ebraeus
Philippines
Guam
American Samoa
Easter Island
C. Conus miliaris
Okinawa
Hawaii
Guam
D. Conus sanguinolentus (and Conus lividus)

Figure 1. Geographic distributions (from Röckel *et al.* 1995) and patterns of genetic subdivision of tropical Pacific *Conus* species; sample locations where $N > 8$ and pairwise estimates of Φ_{ST} were obtained are indicated in figure. Dashed lines indicate geographic distributions of species. Solid lines indicate estimated values of Φ_{ST}; line thickness corresponding to arbitrary cut-offs of Φ_{ST} values (less than 0.05, 0.05–0.1, and greater than 0.1) illustrate inferred levels of genetic differentiation among locations with sample sizes greater than eight individuals as indicated in the figure inset. Locations are grouped by shading to show genetically subdivided groups (i.e., with Φ_{ST} values greater than 0.1). **A,** *Conus chaldaeus*; **B,** *Conus ebraeus*; **C,** *C. miliaris* (based on Duda and Lee 2009); **D,** *C. sanguinolentus*, geographic distribution of *C. lividus* shown with dashed gray lines (see text).

Okinawa (*C. lividus* and *C. sanguinolentus*), Guam (*C. chaldaeus*, *C. lividus* and *C. sanguinolentus*), American Samoa (*C. chaldaeus*, *C. lividus* and *C. sanguinolentus*), French Polynesia (*C. sanguinolentus*), Hawaii (all four species) and Clipperton Island (*C. chaldaeus*). We 'preidentified' specimens of *C. lividus* and *C. sanguinolentus* based on the presence or absence of a pale central band on the last whorl of the shell, respectively. Ultimately, we confirmed or re-evaluated identifications of these two species based on the sequences obtained from these individuals (as described below). The sample size of *C. lividus* was too small to permit phylogeographic investigation of this species and so all analyses proceeded with the other species. We also obtained additional specimens from Clipperton Island from the Invertebrate Zoology collections at the Santa Barbara Museum of Natural History to increase the sample size of *C. ebraeus* from the eastern Pacific that was used in a previous study (*i.e.*, Duda and Lessios 2009).

DNA sequences

We extracted DNA from specimens using the Omega-Biotek EZNA Mollusc DNA kit (Doraville, Georgia, USA). We amplified a region of approximately 650 basepairs (bp) (excluding primers) of the mitochondrial *COI* gene with LCO1490 and HCO2198 primers (Folmer *et al.* 1994) at an annealing temperature of 45°C. We sequenced the amplification products directly in both directions with their original amplification primers at the University of Michigan DNA Sequencing Core facility. We evaluated chromatograms and sequence calls with Sequencher 4.9 (Gene Codes Corporation, Ann Arbor, Michigan, USA) and aligned sequences by eye with Se-Al v.2.0a11 (Rambaut 2002). We also included in our analyses *COI* sequences from three individuals that were preidentified as either *Conus lividus* or *C. sanguinolentus* from American Samoa, French Polynesia and Réunion Island that were gratefully provided by Christopher P. Meyer (National Museum of Natural History) and a previously published sequence from an individual of *C. lividus* from American Samoa (GenBank accession number AY588192, Table 1).

Sequence analyses

We constructed haplotype networks based on analyses of *COI* sequences of *Conus chaldaeus*, *C. lividus* and *C. sanguinolentus* using TCS 1.21 (Clement *et al.* 2000). *COI* sequences from specimens of both *C. lividus* and *C. sanguinolentus* were initially combined; positions of specimens within the resultant network were used to confirm or re-evaluate the identities of these specimens (described in *Results*). Numbers of haplotypes and haplotype diversity of each species and each location were determined with Arlequin version 2.0 (Schneider *et al.* 2000). Modeltest v.3.7 (Posada and Crandall 1998) was used to determine the best model of nucleotide substitution for each species.

Table 1. Genbank accession numbers of *COI* sequences of *Conus* species.

Species	GenBank accession numbers
C. abbreviatus	HQ852701– HQ852725
C. chaldaeus	HQ852592– HQ852682
C. ebraeus	AY588175[1], EF547559–EF547649[2], HQ852683–HQ852700
C. lividus	AY588192[1], HQ852563–HQ852591
C. miliaris	AY588203[1], FJ392914–FJ393023[3], FJ411486–FJ411516[3]
C. sanguinolentus	HQ852472–HQ852562

[1] from Duda and Rolán 2005
[2] from Duda and Lee 2009
[3] from Duda and Lessios 2009

To examine patterns of phylogeography, we estimated Φ_{ST} values for all pairwise comparisons of samples of locations with sample sizes greater than eight individuals with Arlequin. We also used Arlequin to determine if values of Φ_{ST} deviated significantly from the null hypothesis of no differences between locations based on the proportion of 10,100 permutations of haplotypes between locations that gave Φ_{ST} values greater to or equal the observed Φ_{ST} values. Moreover, we conducted an analysis of molecular variance (AMOVA) using Arlequin to examine the partitioning of genetic variance within and among groups of populations (*i.e.*, samples from each location) for each species; group memberships contained samples from locations that exhibited Φ_{ST} values that were less than 0.01 and not significant. For *Conus chaldaeus* this included a combined sample from Guam and Hawaii ('Guam+Hawaii'); for *C. sanguinolentus* it included a combined sample from Okinawa and Guam ('Okinawa+Guam').

We examined the historical demography of populations with analyses of mismatch distributions (Rogers and Harpending 1992, Rogers 1995) of *COI* sequences also with Arlequin. As above, population sets were comprised of samples from different locations that showed no evidence of population structure (*i.e.*, with Φ_{ST} values < 0.01). We compared the mismatch distribution of these populations to expectations for demographic population expansion by examining the sum of squared deviations and Harpending's raggedness index of observed and expected distributions under a model of demographic population expansion with Arlequin. Estimates of times since population expansion (τ, the number of generations scaled by the mutation rate) and population sizes before (θ_0) and after (θ_1) expansion ($\theta = N_e\mu$, where N_e is the effective population size and μ is the mutation rate) and their 95% confidence intervals were also determined with Arlequin. Estimates of τ (*i.e.*, $\tau = 2\mu t$) were converted to absolute time using a *COI* divergence rate of 3.7 substitutions per million year as utilized by Duda and Lessios (2009). We also estimated Tajima's *D* (Tajima 1989) and Fu's *Fs* (Fu 1997) statistics to evaluate demographic histories of populations of these species as well as *Conus miliaris* (these statistics were not originally determined by Duda and Lee 2009) with Arlequin. Significantly negative values of these statistics usually indicate recent population expansion though they can also be evidence of recent selective sweeps (Tajima 1989, Fu 1997).

We also conducted analyses using IMa (Hey and Nielsen 2007) to estimate scaled times of separation of populations, effective population sizes, and directional migration rates where appropriate. Initial runs were started with population samples (as defined above) of *Conus abbreviatus*, *C. chaldaeus*, *C. ebraeus* and *C. sanguinolentus* using initial parameter values suggested in the program manual and the Hasegawa, Kishino and Yano (1985) (HKY) model of nucleotide substitution. For *C. chaldaeus* we ran three independent analyses that included two sets of populations: Clipperton Island and American Samoa, Clipperton Island and Guam+Hawaii, and American Samoa and Guam+Hawaii. Final runs included input parameters that bounded the range of parameter values observed in test runs, a geometric heating scheme with 20 chains, a burn-in of 10^5 steps and at least 10^5 saved genealogies (*i.e.*, at least 10^6 additional steps after burn-in). In addition, we also employed msBayes (Hickerson *et al.* 2007) to test for simultaneous divergence of Indo-West Pacific and eastern Pacific populations of *C. chaldaeus* and *C. ebraeus*. We utilized default bounds for prior distributions of all parameters as generated by the program and evaluated the posterior probabilities of models with one and two divergence times for the population-pairs of these species.

RESULTS

DNA sequences

We recovered *COI* sequences from 91 individuals of *Conus chaldaeus* from Guam (*N* = 24), American Samoa (*N* = 29), Hawaii (*N* = 29) and Clipperton Island (*N* = 9) (Table 2, Fig. 2A); an additional 18 individuals of *C. ebraeus* from Clipperton Island (Table 2, Fig. 2B); 114 individuals that were preidentified as *C. lividus* or *C. sanguinolentus* (Table 2, Fig. 2C) (location information is provided below) (the total number also includes sequences provided by C.P. Meyer and one previously published sequences); and 25 individuals of *C. abbreviatus* from Hawaii (Table 2, Fig. 1E).

The sequences from *Conus lividus* and *C. sanguinolentus* occurred in two main groups in the network; sequences from these clades differed at a minimum of 57 sites (Fig. 2C). Based on the segregation patterns of individuals identified as *C. lividus* and *C. sanguinolentus* among these clades, the majority of the individuals assayed were *C. sanguinolentus* and approximately half of the individuals that were identified as *C. lividus* were actually *C. sanguinolentus*. In total, *COI* sequences were determined from 88 individuals of *C. sanguinolentus* from Réunion Island (*N* = 1) (sequence from C.P. Meyer), Papua New Guinea (*N* = 1), Okinawa (*N* = 14), Guam (*N* = 48), American Samoa (*N* = 1) (sequence from C.P. Meyer), French Polynesia (*N* = 1) (sequence from C.P. Meyer) and Hawaii (*N* = 25) (including one previously published sequence). All locations except French Polynesia contained at least one specimen that was incorrectly identified as *C. lividus*. Because of the small sample size of *C. lividus* (*N* = 27), further analyses were not conducted on samples of this species, but *C. lividus* occurred at all locations examined except for Réunion Island and French Polynesia where only one specimen from each location was available for study. All new sequences (including the three provided by C.P. Meyer) were deposited in GenBank (Table 1).

Table 2. Sample sizes and information on *COI* haplotype diversity of *Conus* species from locations in the tropical Pacific. *N*, sample size.

Sample	*N*	Number of haplotypes	Haplotype diversity (*SE*)
Conus abbreviatus			
Hawaii	25	22	0.990 (0.014)
Conus chaldaeus			
Guam	24	15	0.964 (0.019)
American Samoa	29	17	0.899 (0.047)
Hawaii	29	16	0.923 (0.034)
Clipperton Island	9	6	0.833 (0.127)
Conus ebraeus			
Clipperton Island	28	16	0.947 (0.022)
Indo-West Pacific[1]	80	41	0.942 (0.018)
Conus miliaris[2]			
Easter Island	61	40	0.965 (0.014)
Non-Easter Island	80	74	0.997 (0.003)
Conus sanguinolentus			
Okinawa	14	5	0.791 (0.067)
Guam	48	11	0.764 (0.042)
Hawaii	25	8	0.543 (0.119)

[1] from Duda and Lessios 2009

[2] from Duda and Lee 2009.

Sequence analyses

We detected 37 unique *COI* haplotypes from 91 individuals of *Conus chaldaeus* (Table 2, Fig. 2A). We obtained *COI* sequences from an additional 18 individuals of *C. ebraeus* from Clipperton Island, including several haplotypes that Duda and Lessios (2009) did not previously observe at this location (Table 2, Fig. 2B). We identified 23 unique *COI* haplotypes from 88 specimens of *C. sanguinolentus* (Table 2, Fig. 2C). Haplotype diversity was high for all samples; *C.*

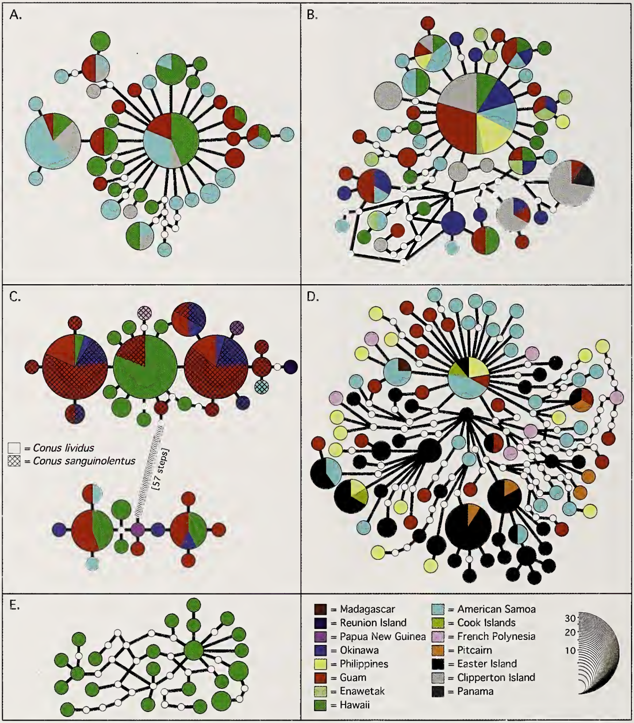
A.
B.
C.
D.
= Conus lividus
= Conus sanguinolentus
[57 steps]
E.
= Madagascar
= Reunion Island
= Papua New Guinea
= Okinawa
= Philippines
= Guam
= Enawetak
= Hawaii
= American Samoa
= Cook Islands
= French Polynesia
= Pitcairn
= Easter Island
= Clipperton Island
= Panama
30
20
10

Figure 2. Haplotype networks of *COI* sequences of tropical Pacific *Conus* species. Each branch represents a single step/substitution between haplotypes; hypothetical haplotypes are indicated with small empty circles. Areas of other circles representing observed haplotypes are proportional to the observed frequency of each haplotype; scale given in lower right. Locations of haplotypes are color-coded and shown as pie diagram within haplotype circles based on legend in lower right. **A**, *Conus chaldaeus*; **B**, *C. ebraeus*; **C**, *C. lividus* and *C. sanguinolentus*; **D**, *C. miliaris* (figure adapted from Fig. 2 in Duda and Lee 2009); **E**. *C. abbreviatus*.

sanguinolentus, particularly at Hawaii, showed the lowest levels of diversity (Table 2). We also observed 22 unique *COI* haplotypes from 25 individuals of *C. abbreviatus* (Table 2, Fig. 2E). For comparison, this information is also presented for the Indo-West Pacific populations of *C. ebraeus* and *C. miliaris* (Table 2, Figs. 2B, 2D).

As observed previously for *Conus ebraeus* and *C. miliaris* (Duda and Lee 2009, Duda and Lessios 2009), Modeltest revealed that the HKY model (Hasegawa *et al.* 1985) was the most appropriate model of nucleotide substitution for the new datasets examined. Nonetheless, the HKY model is unavailable in Arlequin and so we used Tamura-Nei (Tamura and Nei 1993) distances. As discussed previously by Duda and Lessios (2009), Tamura-Nei and HKY distances differ at most at the fifth decimal place and so use of Tamura-Nei distances in Arlequin are unlikely to have noticeably affected our results.

We detected signals of genetic population differentiation for all species that were examined. For *Conus chaldaeus*, significant Φ_{ST} values were detected for comparisons that included samples from (1) American Samoa and Guam and (2) American Samoa and Hawaii (Table 3). Nonetheless, these values (0.063 and 0.065) were much lower than estimates of Φ_{ST} for samples of the other species. Moreover, the Φ_{ST} values from pairwise comparisons that included samples from Clipperton Island, the location with the smallest sample size ($N = 9$), ranged from -0.030 (compared to sample set from American Samoa) to 0.039 (compared to Guam) and 0.058 (compared to Hawaii), but none were significantly different from null expectations. Based on analyses of a larger sample size of *C. ebraeus* from Clipperton Island, we confirmed the previous finding of Duda and Lessios (2009) that this species exhibits a genetic break between populations in the Indo-West Pacific and eastern Pacific. Although our estimates of Φ_{ST} (0.098–0.119) are lower than those estimated previously [0.112 – 0.210; Duda and Lessios (2009)], they still differed significantly from null expectations. Pairwise estimates of Φ_{ST} values of *C. sanguinolentus* were restricted to samples from Okinawa, Guam and Hawaii due to the small sample sizes at other locations. While the estimate of Φ_{ST} between samples from Okinawa and Guam was low (-0.011) and not significantly different from zero, pairwise comparisons between samples from these locations and Hawaii yielded quite large Φ_{ST} values (0.322 and 0.235, respectively) that were significantly greater than null expectations (Table 4).

AMOVAS were conducted for two groups of *Conus chaldaeus* that contained samples from American Samoa and samples from Guam and Hawaii, two groups of *C. sanguinolentus* that contained samples from Hawaii and samples from Okinawa and Guam, and two groups of *C. ebraeus* that included samples from Clipperton Island and samples from Okinawa, Philippines, Guam, American Samoa, Enawetak and Hawaii (data for these latter samples from Duda and Lessios 2009). In all cases, most of the genetic variance was partitioned within populations (*i.e.*, samples from each location) with values of 93.6%, 76.8% and 87.8% recorded for *C. chaldaeus*, *C. sanguinolentus* and *C. ebraeus*, respectively; only minor fractions of the variance were partitioned among populations within groups (0.4%, -0.3% and -0.4%, respectively). AMOVA results showed that 6.0%, 23.5% and 12.6% of the genetic variance was partitioned among groups for *C. chaldaeus*, *C. sanguinolentus* and *C. ebraeus*, respectively.

Table 3. Pairwise Φ_{ST} values for samples of *Conus chaldaeus* from different locations in the tropical Pacific.

	American Samoa	Hawaii	Clipperton
Guam	0.063*	0.004^{NS}	0.039^{NS}
American Samoa		0.064**	-0.030^{NS}
Hawaii			0.058^{NS}

NS, not significant; *, $P < 0.01$; **, $P < 0.005$

Table 4. Pairwise Φ_{ST} values for samples of *Conus sanguinolentus* from different locations in the tropical Pacific.

	Guam	Hawaii
Okinawa	-0.011^{NS}	0.322*
Guam		0.235*

NS, not significant; *, $P < 0.001$

Observed mismatch distributions of *COI* haplotypes corresponded to expectations of a model of recent population expansion for all populations examined, including *C. abbreviatus* from Hawaii, *C. chaldaeus* from American Samoa and combined samples from Hawaii and Guam, *C. ebraeus* from Clipperton Island and *C. sanguinolentus* from Hawaii and combined samples from Okinawa and Guam (Table 5). The 95% confidence intervals of estimates of the time since expansion scaled by mutation rate are broadly overlapping for all populations. Conversion of the values to absolute time provides estimates of times since expansion within the past one million years for most populations (Table 5). The only outlier is the population of *C. ebraeus* at Clipperton Island with an estimated time since expansion that ranged from 400 thousand to two million years ago; the population of this species from the Indo-West Pacific appears to have undergone expansion more recently (20,000–70,000 years ago). In most cases, analyses of Tajima's *D* and Fu's F_S confirmed results from analyses of mismatch distributions (*i.e.*, significantly negative values of these statistics that suggest recent population expansion were observed) (Table 5). Exceptions included the Clipperton Island population of *C. ebraeus* based on Tajima's *D* estimate and the combined samples of *C. sanguinolentus* from Okinawa and Guam for both Tajima's *D* and Fu's *Fs*.

Parameter estimates from the IMa runs are presented in Table 6. In all cases results from multiple runs gave similar results. As indicated, a few of the estimated parameters exhibited incomplete posterior distributions in the analyses, presumably due to lack of sufficient information in the data. Estimates of θ ($\theta = 2N_e\mu$, where N_e = effective population size, μ = mutation rate) are largely comparable to values

Table 5. Mismatch distribution statistics from analyses of patterns of variation of *COI* haplotypes of populations of *Conus* species from the tropical Pacific. *N*, sample size; SSD, sum of squared deviations of observed and expected mismatch under a model of recent population expansion; HRI, Harpending's Raggedness index; τ, time since expansion scaled by the mutation rate (μ) (*i.e.*, $\tau = 2\mu t$); *t*, absolute time in millions of years (my) since expansion based on estimated mutation rate (*i.e.*, converted values of τ); θ_0 and θ_1, initial and current estimates of theta ($\theta = 2N_e\mu$, N_e = effective population size); *D*, Tajima's (1989) *D* statistic; *Fs*, Fu's (1997) F_S statistic.

				Mismatch distribution statistics					
Sample	*N*	SSD	HRI	τ (95% CI)	*t* (my) (95% CI)	θ_0 (95% CI)	θ_1 (95% CI)	*D*	*Fs*
Conus abbreviatus									
Hawaii	25	0.003^{NS}	0.016^{NS}	5.6 (3.0–7.4)	0.8 (0.4–1.0)	0.1 (0.0–2.4)	78 (23–7250)	-1.8*	$-\infty$***
Conus chaldaeus									
Guam+Hawaii	53	0.001^{NS}	0.032^{NS}	2.9 (1.5–3.5)	0.4 (0.2–0.5)	0.0 (0.0–1.5)	4081 (37–10067)	-2.0**	-22.4***
American Samoa	29	0.012^{NS}	0.047^{NS}	3.2 (1.4–5.6)	0.4 (0.2–0.8)	0.0 (0.0–1.3)	14.1 (5.1–6720)	-1.7*	-10.5***
Clipperton	9	0.143^{NS}	0.549*	4.7 (1.9–8.6)	0.6 (0.3–1.2)	0.0 (0.0–4.2)	12.9 (3.9–6609)	-0.9^{NS}	-1.0^{NS}
Conus ebraeus				(1.9–8.6)					
Clipperton	28	0.010^{NS}	0.020^{NS}	8.2 (3.1–13.9)	1.1 (0.4–1.9)	0.0 (0.0–4.4)	11.4 (5.0–173)	0.5^{NS}	-4.5*
Indo-West Pacific[1]	80	0.010^{NS}	0.020^{NS}	1.4 (0.4–5.5)	0.2 (0.1–0.7)	2.8 (0.0–8.4)	1062 (20.9–7586)	-2.3***	-11.2**
Conus miliaris[2]									
Indo-West Pacific (non-Easter Island locations)		0.002^{NS}	0.008^{NS}	5.1 (3.1–10.1)	0.7 (0.4–1.4)	1.5 (0–2.5)	36.9 (15.8–4924)	-2.2***	-12.4***
Easter Island only		0.006*	0.021^{NS}	n/a	n/a	n/a	n/a	-1.8*	-13.1****
Conus sanguinolentus									
Okinawa+Guam	62	0.023^{NS}	0.063^{NS}	3.3 (0.9–7.1)	0.4 (0.1–1.0)	0.0 (0.0–1.8)	4.1 (1.4–2972)	-0.6^{NS}	-3.1^{NS}
Hawaii	25	0.000^{NS}	0.062^{NS}	0.9 (0.0–2.6)	0.1 (0.0–0.4)	0.0 (0.0–0.8)	3.4 (0.4–4779)	-2.1**	-5.4***

NS, not significant; *, $P<0.05$; **, $P<0.01$; ***, $P < 0.005$

[1] from Duda and Lessios 2009, except for Tajima's *D* and Fu's *Fs* which were calculated in the present study

[2] from Duda and Lee 2009, except for Tajima's *D* and Fu's *Fs* which were calculated in the present study

estimated based on mismatch distributions and in most cases suggest that the populations have undergone recent expansion. In addition, except for a few cases, estimates of times of population separation from IMa do not conflict with estimates of times of population expansion based on mismatch distributions (assuming that the separation preceded the expansion). Based on these results, Indo-West Pacific and eastern Pacific populations of *Conus chaldaeus* separated earlier than those of *C. ebraeus*, but the posterior density intervals for these parameters are broadly overlapping (Table 6). Evaluation of the divergence times of the Indo-West Pacific and eastern Pacific populations of *C. chaldaeus* and *C. ebraeus* with msBayes revealed higher posterior probabilities for a model of a single timing of separation for these populations based on categorical regression (0.965) and simple rejection (0.786) than for a model of two divergence times (0.035 and 0.214, respectively).

DISCUSSION

Phylogeographic analyses of mitochondrial sequence data revealed both concordant and discordant patterns among four species of *Conus* in the tropical Pacific (Fig. 1). First, as reported previously for *C. ebraeus* and *C. miliaris* (Duda and Lessios, 2009, Duda and Lee 2009), *C. chaldaeus* and *C. sanguinolentus* showed no genetic population structure throughout large parts of their ranges spanning several thousand kilometers (Figs. 1A, 1D). But despite rather extensive genetic homogeneity across wide geographic areas, at least three of the four species with

Table 6. Estimates of population parameters of *Conus* species based on analyses with IMa. N, number of genealogies examined; θ_i, $2N_e\mu$ (N_e = effective population size, μ = mutation rate; subscripts used for estimates of the populations compared (1 = first listed, 2 = second listed, A = ancestral population)); HPD, 95% highest posterior density interval, except where indicated; t_μ, time of population separation scaled by mutation rate; t, time of separation based on rate calibration; m, migration rate from population scaled by mutation rate where $2N_e m_f = \theta m/2$ (m_f = female migration rate; subscripts used for estimates of the populations compared (1 = first listed, 2 = second listed)).

Population comparison	N (x10^5)	θ_1 (HPD)	θ_2 (HPD)	θ_A (HPD)	t_μ (HPD)	t (my) (HPD)	m_1 (HPD)	m_2 (HPD)
C. abbreviatus	3.0	96.5	n/a	n/a	n/a	n/a	n/a	n/a
Hawaii		(55.5–171)						
C. chaldaeus								
Hawaii+Guam and	17.4	93.8	20.3	0.3	1.3	0.35	1.6	0.1
American Samoa		(27.3–324)	(4.3–88.8)	(0.0–292)	(0.4–9.9)[1]	(0.11–2.7)	(0.0–4.9)	(0.0–5.2)
Hawaii+Guam and	2.9	68.3	9.3	0.3	1.3	0.35	2.7	0.1[2]
Clipperton		(16.8–343)	(0.8–286)	(0.0–406)	(0.6–10.0)[1]	(0.16–2.7)	(0.8–6.0)[1]	n/a
American Samoa	3.0	24.3	6.8	8.3	0.5	0.1	0.0[2]	4.7
and Clipperton		(7.8–461)[1]	(0.0–458)[1]	(0.0–441)[1]	(0.1–10.0)[1]	(0.0–2.7)	n/a	(0.4–6.0)[3]
C. ebraeus								
Indo-West Pacific	1.4	9995[3]	5.0	45.0	0.3	0.08	0.1	14.9
and eastern Pacific		(1555–9995)	(0.0–85.0)	(0.0–135.0)	(0.1–0.5)	(0.03–0.14)	(0.0–4.50)	(0.0–129.9)[1]
C. miliaris[4]								
Easter Island and	50.0	72.6	6128	31.8	1.7	0.45	0.6	1.3
non-Easter Island		(31.1–159.3)[5]	(3677–171,502)[5]	(10.6–64.3)[5]	(1.2–2.5)[5]	(0.32–0.67)[5]	(0.0–2.4)[5]	(0.7–2.1)[5]
C. sanguinolentus								
Hawaii and	30.0	10.7	9.2	0.0	0.7	0.19	0.2	0.0
Okinawa+Guam		(4.3–46.7)[1]	(3.2 – 22.5)	(0.0–44.0)[1]	(0.2–10.0)[1]	(0.05–2.7)	(0.0–2.2)	(0.0–1.6)

[1] distinct peak but posterior density does not reach zero at upper limit of the prior

[2] multiple peaks, HPD not reported

[3] increasing posterior distribution to a plateau

[4] from Duda and Lee (2009)

[5] 90% HPD presented as reported by Duda and Lee (2009)

adequate sample sizes (i.e., not including *C. lividus*) exhibited a uniquely located strong genetic break that occurred at peripheral and isolated locations (Figs. 1B, 1D).

In addition, most populations exhibited recent demographic population expansion that is estimated to have occurred during the past one million years (Table 5). Previous analyses of mismatch distributions of the eastern Pacific population of *Conus ebraeus* rejected the hypothesis of recent population expansion for this population (Duda and Lessios 2009). As shown here, analysis of a larger sample size from Clipperton Island failed to reject this hypothesis and so, as mentioned by Duda and Lessios, interpretations of results from mismatch distributions based on small sample sizes should be considered with caution.

Phylogeographic breaks across the East Pacific Barrier

Analysis of a larger sample size of *Conus ebraeus* confirmed previous interpretations based on the smaller sample size used by Duda and Lessios (2009) that the East Pacific Barrier limits gene flow among populations of *C. ebraeus* in the Indo-West Pacific and eastern Pacific and constitutes a strong phylogeographic break for this species (Fig. 1B). Nonetheless, although the sample size from the eastern Pacific (i.e., Clipperton Island) was small, *C. chaldaeus* did not show this pattern very strongly: estimated Φ_{ST} values among the Clipperton Island sample and Hawaii, Guam and American Samoa samples of this species were all not significantly different from null expectations of a random distribution of haplotypes among locations and the Φ_{ST} value calculated between Clipperton Island and American Samoa samples is negligible (Table 3). Under the assumption that the small sample size was not responsible for failure to reject our null hypothesis of genetic homogeneity across the East Pacific Barrier, the discordant phylogeographic patterns of *C. chaldaeus* and *C. ebraeus*, two sister species that likely share a number of traits aside from similar dispersal abilities, was similar to the disparate patterns observed by Lessios and Robertson (2006) for 20 transpacific fish species that also span the East Pacific Barrier. Because of the small sample size of *C. chaldaeus* from the eastern Pacific, however, these interpretations should be considered with caution until a larger sample size from the eastern Pacific is obtained and examined.

The lack of strong evidence of a coincident genetic break across the East Pacific Barrier for both *Conus chaldaeus* and *C. ebraeus* may have resulted from the following factors: (1) fine scale differences in life history attributes as suggested by Crandall *et al.* (2008) as the possible causes of the discordant phylogeographic patterns of *Nerita albicilla* Linnaeus, 1758 and *Nerita plicata* Linnaeus, 1758 in the Indo-West Pacific, (2) differences in ecological features as suggested by Marko (2004) for *Nucella lamellosa* (Gmelin, 1791) and *Nucella ostrina* (Gould, 1852) in the northeastern Pacific and by Crandall *et al.* (2008) for the two nerite species, or (3) stochasticity in long-distance dispersal events or local extinctions as suggested for discordant patterns of four closely related Indo-West Pacific sea urchin species (*Echinometra*) by Palumbi *et al.* (1997) and 20 transpacific fish species by Lessios and Robertson (2006). *C. chaldaeus* and *C. ebraeus* exhibit similar life histories (Kohn and Perron 1994). In addition, although *C. chaldaeus* and *C. ebraeus* show slight differences in feeding ecologies (Kohn 1959, Kohn and Orians 1962), how these differences would have contributed to the discordant phylogeographic patterns in light of the nearly identical distributions of these species is difficult to interpret. We favor the explanation that stochasticity in long-distance dispersal or population extinction is responsible for the observed patterns. Under this scenario, *C. ebraeus* has had a longer history in the eastern Pacific than *C. chaldaeus* (due to an earlier colonization or massive dispersal event in *C. ebraeus* or a more recent extinction of *C. chaldaeus*) or only *C. chaldaeus* has experienced recent gene flow across the East Pacific Barrier. Although the 95% highest posterior density intervals are wide and some analyses failed to provide appropriate estimates of parameters, results from IMa suggest that the separation of Indo-West Pacific and eastern Pacific populations of *C. ebraeus* is actually more recent (80,000 years ago) than *C. chaldaeus* (350,000 years ago) and that migration rates are higher for populations of *C. ebraeus* (Table 6). Moreover, our data support a simultaneous divergence of Indo-West Pacific and eastern Pacific populations of these species based on model-testing with msBayes. Thus, neither of these hypotheses is supported by our data and we suspect that the sample size of *C. chaldaeus* from the eastern Pacific is perhaps too small to appropriately evaluate its history and degree of differentiation from populations in the Indo-West Pacific.

Phylogeographic patterns in the western and central Pacific

Similar to results observed for these four *Conus* species, many other demersal species with high dispersal potential do not exhibit genetic population structure within large parts of their ranges in the tropical Pacific. These include other gastropods (*Nerita albicilla* and *Nerita plicata*, Crandall *et al.* 2008), and various echinoderms (*Diadema paucispinum* A. Agassiz, 1863 and *Diadema savignyi* Michelin, 1845 Lessios *et al.* 2001; *Linckia laevigata* Linnaeus, 1758, Williams *et al.* 2002; *Tripneustes* species, Lessios *et al.* 2003). The concordance of these results suggests that possession of a relatively long duration planktonic larval phase enhances gene flow or that these species all underwent recent expansion throughout this region and there has not been enough time or local population sizes have been too large for genetic drift to cause differentiation at local scales. On the other hand, a number of tropical Pacific species with high dispersal potential exhibit genetic differentiation in this region, including several molluscs (three *Tridacna* species, Benzie and Williams 1997), several sea urchins (four *Echinometra* species, Palumbi *et al.* 1997; *Eucidaris metularia* Lamarck, 1816, Lessios *et al.* 1999), a lancelet (*Asymmetron lucayanum* clade B Andrews, 1893, Kon *et al.* 2006), and multiple fish species (*Chanos chanos*, Winans 1980 and Ravago-Gotanco and Juinio-Meñez 2004; *Dascyllus trimaculatus* Rüppell, 1829, Bernardi *et al.* 2001; *Acanthurus triostegus* Linneaus, 1758, Planes and Fauvelot 2002; *Chlorurus sordidus* Forsskål, 1775, Bay *et al.* 2004). Although these phylogenetically disparate taxa likely exhibit differences in their potential for dispersal in terms of the lengths of their planktonic larval periods, the contrasting patterns of genetic population structure may be due to the stochasticity in long-distance dispersal or different ecological attributes as discussed above for transpacific species.

Among the four *Conus* species that have been examined to date, the greatest signal of a genetic break occurs for *C. sanguinolentus* at Hawaii. The density of our sampling of *C. sanguinolentus* in the tropical Pacific is poor and we did not include any samples from locations between Guam and Hawaii (*e.g.*, from the Marshall Islands) or from regions in the South Pacific (*e.g.*, American Samoa). Thus, the location of the break at Hawaii itself may be inaccurate and the range of this genetically differentiated population may actually encompass a larger geographic area. Nonetheless, the geographic isolation of the Hawaiian Archipelago in the tropical Pacific at least associates a mechanism (*i.e.*, reduced gene flow at an isolated location) with the hypothesis that the break occurs uniquely at Hawaii. Also, the pattern observed for *C. sanguinolentus* contrasts quite strongly with the lack of genetic differentiation of samples of both *C. chaldaeus* and *C. ebraeus* at this location. Moreover, the Φ_{ST} values that are associated with pairwise comparisons of samples of *C. sanguinolentus* at Hawaii and other locations (0.235–0.322; Table 3) are at least 1.7 times greater than values associated with samples of *C. miliaris* at Easter Island and other locations in the Indo-West Pacific (0.072–0.137; Duda and Lee 2009) and samples of *C. ebraeus* in the eastern Pacific and Indo-West Pacific (0.098–0.119). In addition, these samples also exhibit the lowest level of haplotype diversity for all samples that were examined (Table 2) and results from mismatch distribution analyses suggest that this population expanded more recently than other populations of *Conus* in the tropical Pacific, including the Hawaiian endemic *C. abbreviatus* (Table 5). Together these results suggest that *C. sanguinolentus* at Hawaii is isolated from other populations in the tropical Pacific and that it may have undergone a recent founder event with subsequent expansion at this location. Clearly, a much greater density of samples from the tropical Pacific and analyses of additional loci would help to more precisely identify the location of the genetic break as well as the history of its formation.

Inferences from the demographic histories of populations

All populations examined exhibited patterns of genetic variation that suggest recent demographic expansion within the past one million years, including the Hawaiian endemic *Conus abbreviatus* (Table 5). The nerite species examined by Crandall and coworkers (2008) show this same timing for their expansion as well. The coincidence of these timing suggests a common explanation for the demographic expansion such as expansion of suitable habitats caused by episodes of low sea-level stands during the Pleistocene (Paulay 1990).

If population sizes are large enough to limit the effects of genetic drift, the lack of genetic population structure over large parts of the ranges of tropical Pacific species may have stemmed largely from recent spatial expansion throughout this region that was coupled with the demographic expansion and may not reflect high rates of contemporary gene flow in these species. In addition, although the potential for extensive larval transport could certainly have facilitated expansion throughout large regions of the tropical Pacific in the past, it may only have had minor homogenizing effects on geographically discrete populations after the spatial expansion took place. Population differentiation at particular geographic locations in the tropical Pacific, as observed for these *Conus* species, might simply reflect the lack of recruitment to these areas during past broad geographic expansions of these species. Because of the isolated and peripheral nature of the locations where genetic differentiation was observed, the absence of recruitment to these areas is likely to have resulted from the failure of sufficient numbers of larvae to reach these locations. Nonetheless, if populations at these locations are subject to distinct local selection pressures, invading larvae from elsewhere may not have been able to successfully recruit.

CONCLUSIONS

The phylogeographic patterns of four *Conus* species in the tropical Pacific essentially reiterate the patterns observed for a number of phylogenetically disparate species in this region, including other species of molluscs, echinoderms and fishes. In particular, while some sets of species exhibit similar genetic population structures and patterns of population differentiation, few consistent trends are apparent. Nonetheless, dispersal potential clearly plays an important role in population differentiation at broad scales, especially along the continuum from low to high dispersal species. Like other species in the tropical Pacific, four broadly-distributed *Conus* species with high potential for dispersal via planktonic larvae are genetically homogeneous over large parts of their distributions and in one case (*i.e.*, *C. chaldaeus*) also perhaps across a strong barrier to dispersal. This genetic homogeneity either resulted from a recent spatial expansion of these species in large regions of the tropical Pacific or high levels of contemporaneous gene flow among broadly separated locations. The differences in the locations of the genetic breaks in these species, however, suggest a strongly stochastic nature of dispersal. Although sporadic long-distance dispersal may eventually maintain genetic homogeneity across large parts of the ranges of species in the tropical Pacific, it may too allow for populations to undergo genetic differentiation at isolated locations that could ultimately lead to species formation.

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Unraveling cryptic diversity in the Indo-West Pacific gastropod genus *Lunella* (Turbinidae) using elliptic Fourier analysis*

Suzanne T. Williams[1], Andie Hall[1] and Piotr Kuklinski[1,2]

[1] Natural History Museum, Zoology Dept, Cromwell Road, London SW5 5BD, United Kingdom

[2] Institute of Oceanology, Polish Academy of Sciences, Sopot, Poland

Correspondence, Suzanne T. Williams: s.williams@nhm.ac.uk

Abstract. Morphological variation in shell shape was quantitatively evaluated among newly identified cryptic species in two species complexes (*coronata* and *cinerea* groups) belonging to the gastropod genus *Lunella* (Turbinidae) using elliptic Fourier descriptors (EFDs). Sympatric species of the *coronata* group show greater phenotypic diversity than the entirely allopatric *cinerea* group. We suggest that if morphological divergence is driven most strongly in sympatry, cryptic species are more likely to occur in taxa with allopatric speciation. Discriminant analysis showed that one species (*L.* aff. *coronata* ophiolite) was significantly different and could be distinguished from its two sister species (*L. coronata* (Gmelin, 1791), *L.* aff. *coronata* Oman) by shell outline with a high degree of success. In pairwise analyses two pairs from the *cinerea* group were also significantly different and species could be determined with a modest degree of success: *L.* aff. *cinerea* Fiji with *L.* aff. *cinerea* SW Pacific and *L.* aff. *cinerea* Indian Ocean with *L.* aff. *cinerea* central Indo-West Pacific (cIWP). The remaining species could not be accurately identified by shell shape alone in this study. We also used a combination of shell shape and shell sculpture to determine that the coronate form of *L. coronata*, which occurs along the east coast of Africa and along the Arabian Peninsula into India, corresponds to *L. coronata sensu stricto*. We were unable to assign either a holotype for *L. cinerea* (Born, 1778) or a figured specimen of *L. versicolor* (Gmelin, 1791) to genetic lineages with any statistical confidence, although the shell outline of the former is most similar to *L.* aff. *cinerea* cIWP and the latter to *L.* aff. *cinerea* SW Pacific. Finally, using morphometrics, in combination with other shell characters, two fossil species, one from the *cinerea* group from the Pleistocene and one Japanese species from the Eocene, were compared with extant species. Using this method in combination with other characters, we suggest that the Japanese *L. kurodai* Itoigawa, 1955 is most likely a member of the *coronata* group and the Pleistocene *cinerea* fossil is most similar to *L. cinerea* cIWP.

Key words: morphometrics, outline analysis, systematics, taxonomy, non-adaptive radiation, cryptic species

It is crucial for our understanding of the marine environment that we can accurately determine levels of biodiversity. Within some habitats, such as the deep-sea, our knowledge of biodiversity is poor, but even in better-known habitats diversity is still often underestimated. Many genetic studies have shown that even apparently well-known marine groups can harbor high levels of cryptic species (*e.g.* Knowlton 1993). The identification of cryptic species has important ramifications for both fisheries and conservation of species that are exploited for their shell or meat. Recent studies of other organisms have shown that treating several cryptic species as a single management unit for fisheries can lead to over fishing of one species and subsequently an imperiled conservation status (*e.g.* Iglésias *et al.* 2009, Dulvy and Reynolds 2009). The discovery that a single described species actually represents several cryptic species may also create nomenclatural difficulties. Many historical type specimens have little or no locality data or may even have misleading information. There is usually no soft tissue, so identifications must rely entirely on shell characters. The presence of cryptic species leads to the problem of determining to which newly recognized species the name borne by the type specimen should be applied.

Many cryptic taxa are first identified using molecular techniques and indeed there has been some (controversial) suggestion that DNA-taxonomy might replace morphology-based taxonomy (*e.g.* Hebert *et al.* 2003). More often DNA sequences are seen as another marker for helping to identify species, albeit an important one (*e.g.* Puillandre *et al.* 2010). However, for comparison with fossils and historical types, it is critical to identify morphological characters that can distinguish species. In the case of some cryptic species, such characters may be easily recognized with hindsight. The 'cryptic' nature of such species reflects more the lack of detailed taxonomic studies or a change in the perception of which characters are 'important'. In some cases, however, it can be very difficult to distinguish among species, even those with deep genetic divergences (*e.g.* Reid *et al.* 2006, Reid 2007), and more complex character analyses involving the use of morphometrics may be helpful.

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Biological shapes are complex and shape analysis captures a much higher proportion of the morphological information than biometric methods based on discrete linear and angular measurements (Crampton 1995, Van Bocxlaer and Schultheiß 2010). Outline approaches offer an alternate method for analyzing shape variation and have been applied successfully to a range of organisms. Elliptic Fourier descriptors (EFDs) (Kuhl and Giardina 1982) can delineate any form of two-dimensional shape with a closed contour. Elliptic Fourier analysis (EFA) has been effectively applied to the analysis of various biological shapes in a variety of organisms including molluscs (*e.g.* Puillandre *et al.* 2009, Puillandre *et al.* 2010, de Aranzamendi *et al.* 2010, Costa *et al.* 2010, Van Bocxlaer and Schultheiß 2010, González-Wevar *et al.* 2011). Moreover EFA has several important advantages over alternate morphometric analyses (Iwata *et al.* 1998). Mathematical normalization of EFDs means that they are invariant with respect to scale, rotation or location, and starting position of chain coding contour tracing. It is also possible to visualize shape variation that may be difficult to describe verbally (Hiraoka and Kuramoto 2004). Finally, it does not require landmarks, which may be difficult to use in groups where few homologous landmarks can be identified reliably (*e.g.* Marko and Jackson 2001, de Aranzamendi *et al.* 2010, Van Bocxlaer and Schultheiß 2010). One recent study has shown that EFA is superior to landmark analysis in taxa where most unambiguously assignable landmarks are located along the outline of the structure, but homology is uncertain (Van Bocxlaer and Schultheiß 2010; see also Puillandre *et al.* 2009). (However see Papadopoulos *et al.* 2004, Conde-Padin *et al.* 2007, Cunha *et al.* 2008, for some examples where landmark analysis has successfully captured shape variation in mollusk shells).

The turbinid gastropod genus *Lunella* Röding, 1798 occurs, often in large numbers, on rocky shores in the intertidal zone throughout the Indo-West Pacific. Although a well-known genus, *Lunella* has not been the focus of any detailed monographic studies, however recent work has resulted in many changes to its taxonomy. Five species were recognized within the subgenus *Lunella* in a recent guide to the genus *Turbo* Linnaeus, 1758 (Alf and Kreipl 2003). *Lunella* was then recognized as distinct from the genus *Turbo* and raised to generic rank by Williams (2007). Three species previously assigned to *Turbo* were reassigned to *Lunella* (*L. smaragdus* (Gmelin, 1791), *L. torquata* (Gmelin, 1791) and *L. undulata* (Gmelin, 1791); Williams 2007) and two new species were described in recent years, *L. jungi* (Lai, 2006) and *L. ogasawarana* Nakano, Takahashi and Ozawa 2007, making ten nominal species (Alf and Kreipl 2011). Genetic studies have since identified eight cryptic lineages making a total of 18 putative species in this genus (Williams *et al.* 2011; a species-level phylogeny of 17 *Lunella* species is given in Fig. 1; see also Fig. 2).

In Williams *et al.* (2011) the list of putative species was given as a working hypothesis, which needs to be confirmed by more detailed studies. This study aims to determine whether shell shape is a useful character to distinguish genetically identified clades. We examine the utility of elliptic Fourier analysis (EFA) of shell outline for 1) distinguishing newly identified cryptic species within *Lunella*, 2) determining to which of the cryptic species the available type specimens belong, and 3) to determine similarities between fossil specimens and extant taxa.

MATERIALS AND METHODS

Samples

Many of the genetic samples of *Lunella* species used in previous studies (mainly Williams *et al.* 2011) were from specimens with cracked shells (which results in increased ethanol penetration and better DNA preservation) or tissue samples lacking shell vouchers. Therefore, few complete shells were available for morphological examination. Of those that were whole, a proportion of shells from the *coronata* group were covered in epibiotic fauna (mostly barnacles), which would affect outline analysis. Finally, to avoid problems associated with allometric changes during ontogeny we excluded juveniles. We were unable to determine which specimens had reached maturity from shell characters alone, so we used size as a proxy, as has been done in other morphometric studies (Conde-Padín *et al.* 2007, Cunha *et al.* 2008). The species *Lunella viridicallus* (Jousseaume, 1898) although recognized as a valid species in Alf and Kreipl (2003) was not included as we had no confirmed specimens of this species and its status and phylogenetic placement had not been tested by molecular methods.

Names used in this study are based on Williams *et al.* (2011). Additional names are available in the literature that may be applied to some of the cryptic lineages, but these nomenclatural decisions await a more detailed study (STW unpubl. data).

Type specimens

Type specimens of *Lunella coronata* (Gmelin, 1791) and *L. cinerea* (Born, 1778) were included in our analyses. We were able to examine and photograph the holotype of

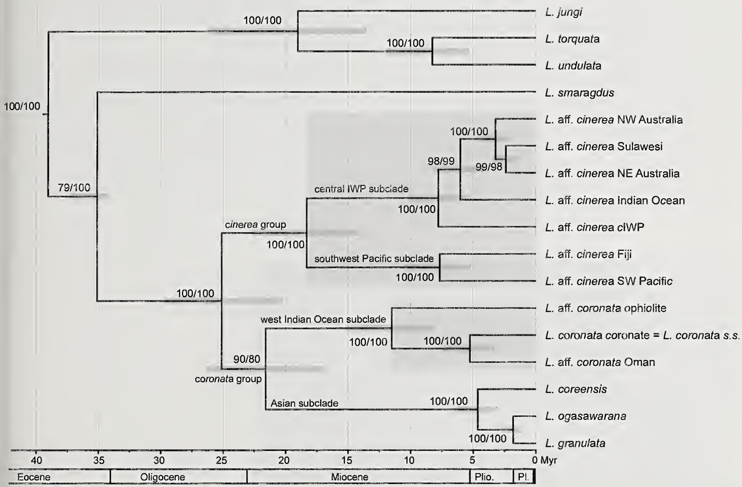
L. jungi
L. torquata
L. undulata
L. smaragdus
L. aff. cinerea NW Australia
L. aff. cinerea Sulawesi
L. aff. cinerea NE Australia
L. aff. cinerea Indian Ocean
L. aff. cinerea cIWP
L. aff. cinerea Fiji
L. aff. cinerea SW Pacific
L. aff. coronata ophiolite
L. coronata coronate = L. coronata s.s.
L. aff. coronata Oman
L. coreensis
L. ogasawarana
L. granulata
100/100
100/100
100/100
79/100
100/100
98/99
99/98
central IWP subclade
100/100
cinerea group
100/100
southwest Pacific subclade
100/100
100/100
west Indian Ocean subclade
100/100
100/100
90/80
coronata group
Asian subclade
100/100
100/100
40
35
30
25
20
15
10
5
0 Myr
Eocene
Oligocene
Miocene
Plio.
Pl.

Figure 1. Chronogram for *Lunella* based on Bayesian analysis of concatenated sequence from 28S rRNA, *COI*, *12S* rRNA and *16S* rRNA genes using BEAST (modified from Williams *et al.* 2011). Clades with cryptic species are indicated by grey boxes. Branch lengths are proportional to time and scale in millions of years (Myr) is given below. Tree is a maximum clade credibility tree with median node heights based on 6,500 trees. Horizontal grey bars correspond to 95% highest posterior density (HPD) interval for node heights (ages). Note that the 95% HPD is not shown for the root. Support values are posterior probabilities (*PP*) from BEAST/*BEAST (see Williams *et al.* 2011 for details).

Lunella coronata (Zoological Museum, State Natural Museum, University of Copenhagen, Denmark; registration: ZMUC-GAS-252; see shell in Fig. 3). The holotype of *L. cinerea* is in the Naturhistorisches Museum Wien (Vienna, Austria; registration: NHMW Mollusca 14338). Examination of photos of the holotype used in subsequent analyses suggests that the outer edge of the shell has been filed smooth. This is confirmed by the NHMW Curator of Molluscs (A. Eschner, pers. comm. 2010), who also suggested that the shell has been polished.

The type species of the genus *Lunella* is *L. versicolor* (Gmelin, 1791) by subsequent designation of Powell (1937). Earlier designations (*e.g.* by Fischer 1873; also of *L. versicolor*) are not normally considered valid, as species are listed as 'examples' rather than type species (P. Bouchet, *pers. comm.* 2011). *Lunella versicolor* is generally thought to be synonymous with *L. cinerea*. To test this assumption, we wanted to include the type specimen in the analyses. Unfortunately, although Cernohorsky saw the *L. versicolor* type in the Denmark museum in 1974 (Cernohorsky 1974), it was not found during a recent examination of the collection (O. Tendal, *pers. comm.* 2010), so we based our analysis on the figure Gmelin refers to in Martini and Chemnitz (1781: pl. 180, Figs 1740–1741 – note that Figure 1741 is incorrectly labeled as figure 1742). In the plate of figures the shell is drawn approximately the same size as *Turbo mespilus* Gmelin, 1791, for which the type specimen is available, so we assumed a similar size for *L. versicolor*. Additional, smaller specimens referred to on the same plate as β (Fig. 1747a,b) and γ varieties of *T. versicolor* (Fig. 1747c,d) are likely a different species and were not included.

DNA Extraction, Amplification and Sequencing

In order to increase sample sizes for both the genetic and morphometric analyses, mitochondrial cytochrome oxidase

(*COI*) sequences were obtained for 68 additional specimens from the *cinerea* and *coronata* groups. DNA was extracted using high-throughput technology with automated preparation of samples using a Qiagen Biosprint 96, which uses magnetic-particle technology. The Qiagen DNA Plant Kit was used following manufacturer's instructions. Part of the mitochondrial cytochrome oxidase (*COI*) gene was amplified using primers and protocols in Williams *et al.* (2011). Double-stranded sequences were edited using Sequencher (v. 4.6, Gene Codes Corporation, Ann Arbor, Michigan). All new sequences have been deposited in GenBank (accession numbers HQ681125–92). Alignment of *COI* was unambiguous, requiring no insertions and was done by eye in MacClade (v 4.08 OSX; Maddison and Maddison 2003), checking amino acid translations.

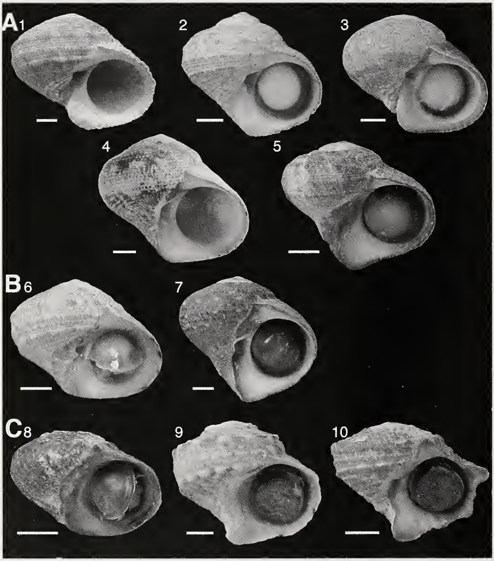

Figure 2. **A**, Species in Dataset A. 1: *Lunella* aff. *cinerea* NW Australia; 2: *L.* aff. *cinerea* Sulawesi; 3: *L.* aff. *cinerea* NE Australia; 4: *L.* aff. *cinerea* Indian Ocean; 5: *L.* aff. *cinerea* cIWP. **B**, Species in Dataset B. 6: *L.* aff. *cinerea* Fiji; 7: *L.* aff. *cinerea* SW Pacific. **C**, Species in Dataset C. 8: *L. coronata* aff. ophiolite; 9: *L. coronata* coronate; 10: *L. coronata* aff. Oman.

Phylogenetic analysis and species assignment

Delimitation of species was undertaken in a previous study (Williams *et al.* 2011). New, as yet unnamed species are referred to in this paper as *Lunella* aff. *cinerea* or *L.* aff. *coronata* with a distinguishing descriptor following (*e.g.* ophiolite) to indicate that these species are related, but distinct from the named taxon. In the *cinerea* group, all species are described as *L.* aff. *cinerea* as it was not possible to determine which species corresponds to *L. cinerea s.s.*

In Williams *et al.* (2011) species were shown to fall into reciprocally monophyletic clades referred to in that paper as evolutionary significant units (ESUs). To confirm their phylogenetic placement in species and clades identified in Williams *et al.* (2011), new sequences from this study were combined with previously published sequences (from Williams and Ozawa 2006, Nakano *et al.* 2007, Williams 2007, Williams *et al.* 2011) and analyzed using Bayesian inference (total $N = 305$). Some new specimens had damaged shells, but were included to test distribution ranges. Species from the *torquata* group, *L. smaragdus* and 12 turbinid species were used as outgroups as in previous studies (Williams *et al.* 2011). The most appropriate nucleotide substitution model was the Hasegawa-Kishino-Yano model (Hasegawa, Kishino and Yano 1980) with invariant sites and gamma distributed rate variation among sites (HKY+I+G) as determined by MrModelTest using the hierarchical likelihood-ratio test (v 2.1, J. Nylander, www.ebc.uu.se/systzoo/staff/nylander.html).

A phylogeny was produced for 305 *COI* sequences using Bayesian inference as implemented in MrBayes (v. 3.2, Huelsenbeck and Ronquist 2001). Analyses were run for 25,000,000 generations with a sample frequency of 10,000. The first 501 trees were discarded, so that 2,000 trees were accepted for each run after likelihood values had reached a plateau. The datasets were analyzed in two independent runs, and the final tree was computed from the

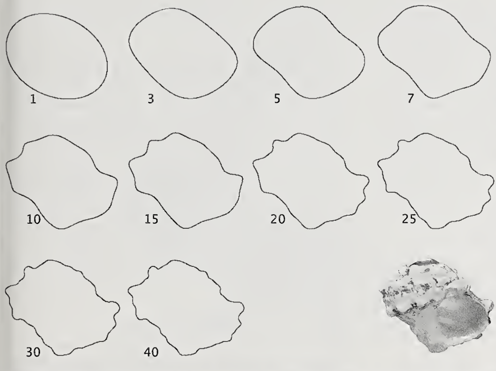

Figure 3. Outline reconstructions of the holotype of *Lunella coronata*, figured, with increasing number of harmonics.

combination of accepted trees from each run (a total of 4,000 trees). Convergence between the two runs was tested by examining the potential scale reduction factors (PSRF) and standard deviation of split frequencies.

Additional, unpublished sequences for one specimen each of *Lunella* aff. *cinerea* Sulawesi and *L. cinerea* aff. Oman and two specimens of *L.* aff. *cinerea* Indian Ocean kindly provided by Tomo Nakano were not included in our tree, but were analyzed separately to confirm specific status and to provide new distribution data (Fig. 4).

Morphometric analyses

Samples used in morphometric analyses ($N = 115$) included a subset of samples used in genetic studies. In some species where sample sizes were low, additional dry specimens from the Natural History Museum, London (NHMUK) collection were included if these were from a locality already included in genetic analyses (when species are allopatric) or identification was certain.

Five different datasets were analyzed. Three of these were to investigate whether elliptic Fourier analysis could be used to distinguish cryptic species and focused on those clades with species complexes (Fig. 1). To reduce the number of species being compared, the *cinerea* group was dealt with in two parts. The two groups were the highly divergent central IWP subclade (five species; dataset A) and the southwest Pacific subclade (two species; dataset B). These groups were chosen because they are genetic clades of approximately similar age with allopatric ranges (given sampling to date) (Fig. 1 and maps in Fig. 4). The third dataset was the west Indian Ocean subclade of the *coronata* group (three species; dataset C). It included both cryptic species and the type specimen of *Lunella coronata* and so was analyzed both to distinguish between species and to identify the species most similar to the holotype. The last two datasets were analyzed solely to determine the species most similar to an 'unknown.' Unknown specimens were fossils (two species) or types (of three species). One analysis was performed including all *cinerea* group species to compare with two type specimens and a Pleistocene fossil from Java belonging to the *cinerea* group (dataset D). Finally, all specimens from both the *cinerea* and *coronata* groups were used to determine which species were most similar to two samples of the fossil species *L. kurodai* Itoigawa, 1955 (dataset E).

Digital images of the shells were acquired on a black background. Shells were always positioned in the same orientation and maintained in position with Blu-tack. A ruler was included for calibration purposes. Photos were edited in Adobe Photoshop to remove background detail and to prepare a standard sized scale (a white rectangle 10 mm x 5 mm) and then converted to MS Windows bitmap. A series of outline shape analyses were performed using the SHAPE software package (v. 1.3; Iwata and Ukai 2002). Digital images were binary-encoded to produce a closed curve and the contour of each shell was traced automatically and expressed as chain-code (Freeman 1974) using ChainCoder (in SHAPE). Each contour was represented as a sequence of x and y coordinates of ordered points that were measured counterclockwise from an arbitrary starting point (Yoshioka *et al.* 2004). The coefficients of elliptic Fourier descriptors (EFDs) were calculated by discrete Fourier transformation from chain-code (Kuhl and Giardina 1982) using Chc2Nef (in SHAPE). Fourier reconstructions using increasing numbers of harmonics, compared to the original outline, was used to estimate that 25 harmonics were sufficient to reconstruct the outlines with high accuracy (Fig. 3), resulting in 100 coefficients of normalized EFDs. Less harmonics were needed to describe simpler outlines of some *cinerea* group species, but we analyzed all data using the same protocol so that datasets could be combined in Dataset E. EFDs were mathematically normalized to be invariant with respect to scale, rotation or location and starting position of chain coding contour tracing in accordance with the procedures suggested by Kuhl and Giardina (1982). Normalization was based on the longest radius and outlines were aligned manually in Chc2Nef. A principal component analysis of the variance-covariance matrix from the EFDs coefficients was performed using PrinComp (in SHAPE) to summarize the information contained in the normalized EFD coefficients (Rohlf and Archie 1984). The shape variation accounted for by each principal component for the first thee datasets (A–C) was visualized using PrinPrint (in SHAPE) using the method of Furuta *et al.* (1995) (Fig. 5). The variation observed between the first two principal components was plotted using MS Excel (Fig. 6).

We used the Multivariate Discriminant Analysis function in Statistica (v.8; StatSoft Inc. 2008) on all principal components (PCs) that explained at least 5% of the variation. We used multivariate analysis of variance (MANOVA) and discriminant analyses to determine 1) whether cryptic species were significantly different from each other (datasets A–C), 2) the proportion of specimens belonging to cryptic species that could be accurately classified *post hoc* (datasets A–C), and 3) to classify unknowns that were not classified *a priori* (holotypes and fossils; datasets C–E). For each species group centroids were determined (the location of the point that represents the means for all variables in the multivariate space). For each specimen Mahalanobis distances were calculated from each of the group centroids. Each specimen was classified as belonging to the group to which it was closest, with the smallest Mahalanobis distance. The posterior probability (*PP*) that an unknown belongs to a particular group is inversely related to the Mahalanobis distance from that group centroid and is the probability, based on our knowledge of the values of other variables that the respective sample belongs to a particular group. We also performed jackknifed calculations (leave-one-out) using PAST (Hammer *et al.* 2001). We looked for significant differences among species by calculating Hotelling's *t* test with jackknife correction and *post hoc* classifications among pairwise comparisons of species using PAST.

We checked for deviation from normality (by visual comparison of data with a normal curve). We were not able to test multivariate normality. Homogeneity of variances was confirmed using Levene's (1960) test. The absence of a positive correlation between means and variances across groups was confirmed by visual examination of plots in Statistica. In all cases where multiple tests were performed we applied the sequential Bonferroni procedure. To check whether allometric effects were contributing to PCs, shell area (mm^2) was estimated using ChainCoder as a proxy for shell size and multiple and simple linear regressions were calculated between area and each PC using DataDesk XL add-in to MS Excel.

RESULTS

Molecular analyses

Average potential scale reduction factor for parameter values was 1.000, with a maximum of 1.012. Average standard deviation of split frequencies for partitions with frequency > 0.10 in at least one run approached zero (0.000031). Together these results suggest that Bayesian runs had converged (Gelman and Rubin 1992).

The *COI* tree (Fig. 7) was essentially identical to that for *COI* in Williams *et al.* (2011), recovering all the clades and the four major groups discussed in that paper. All new samples were unambiguously assigned to species and clades identified in Williams *et al.* (2011). The addition of new samples did not recover any instances of sympatry among species of the *cinerea* group, which is entirely allopatric with sampling to date.

Characterization of shell shape variables

In every dataset, the first PC explained more than half of the total variation, with the second explaining between 12.1% and 22.6%. Eigenvalues and contributions of each PC for each dataset are given in Table 1. In general, few PCs were needed to explain more than 95% of all variation with a maximum of nine needed for dataset E. Only the first three PCs were used in further analyses, as these each account for more than 5% of the variation. The shell shape variation represented by the first three PCs is visualized by outline drawings for the three datasets involving cryptic species (A–C; Fig. 5).

Datasets showed no significant violation of the assumptions for discriminant analysis. Levene's test for homogeneity of variance was non-significant for all datasets (using Sequential Bonferroni) except Dataset E and there was no apparent significant correlation between means and variances of the first three PCs across groups (excluding Dataset B, which had only two points). Visual inspection of graphs suggest that data are approximately normally distributed allowing for small sample sizes. Results of MANOVA tests using the PCs (Table 1) showed that some cryptic species could be distinguished on the basis of shell outline (datasets A–C). One-way ANOVA tests were significant for PC 2 in Datasets B and C (Table 2).

Distinguishing cryptic species

Significant differences were observed among the species in the *Lunella cinerea* central IWP subclade using MANOVA (Table 1). One species when combined with all others in Dataset A was classified *post hoc* with a modest degree of success using the first three PCs. *Lunella* aff. *cinerea* cIWP (Philippines, Japan, Sulawesi, Malaysia) was identified correctly 100% of the time (although only 70% when jackknifed) (Table 3). In pairwise analyses using Hotelling's *t* test it was

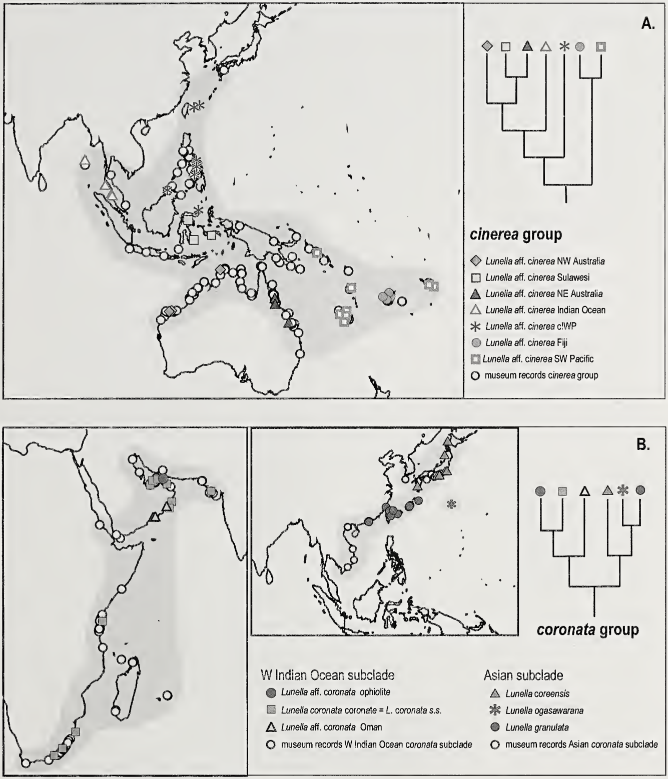
A.
cinerea group
◇ Lunella aff. cinerea NW Australia
□ Lunella aff. cinerea Sulawesi
▲ Lunella aff. cinerea NE Australia
△ Lunella aff. cinerea Indian Ocean
✱ Lunella aff. cinerea cIWP
● Lunella aff. cinerea Fiji
▣ Lunella aff. cinerea SW Pacific
○ museum records cinerea group
B.
coronata group
W Indian Ocean subclade
● Lunella aff. coronata ophiolite
■ Lunella coronata coronate = L. coronata s.s.
△ Lunella aff. coronata Oman
○ museum records W Indian Ocean coronata subclade
Asian subclade
▲ Lunella coreensis
✱ Lunella ogasawarana
● Lunella granulata
○ museum records Asian coronata subclade

Figure 4. Distribution maps for **A**, *cinerea* group and **B**, *coronata* group showing distributions of cryptic taxa based on genetic samples. Grey shading indicates approximate range of each species complex. Open circles show the location of museum samples examined. Museum records have not been identified to species. Colored icons indicate ESUs (according to keys in figures). Note that branch length has no meaning in cladograms. Slightly modified from Williams *et al.* (2011) to include new data from this study and unpublished sequences from Tomo Nakano.

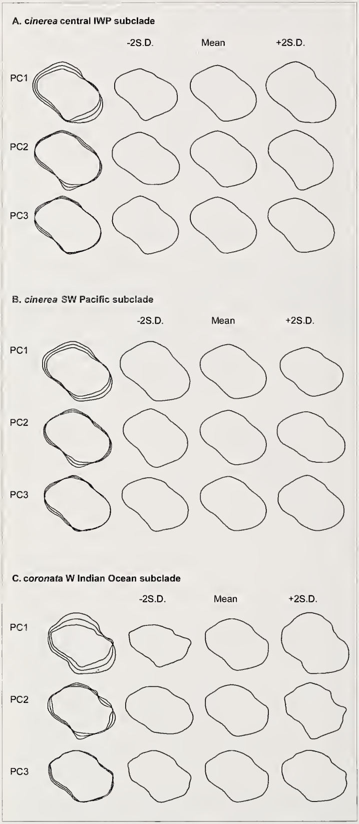
A. cinerea central IWP subclade
-2S.D.
Mean
+2S.D.
PC1
PC2
PC3
B. cinerea SW Pacific subclade
-2S.D.
Mean
+2S.D.
PC1
PC2
PC3
C. coronata W Indian Ocean subclade
-2S.D.
Mean
+2S.D.
PC1
PC2
PC3

Figure 5. Variation in shell shape expressed by the first three principal components (PC) for three datasets (A–C). Outlines are the mean shell shape, and shell shape plus or minus two standard deviations (*S.D.*). The first column shows these three shapes overlapping.

significantly different from the Indian Ocean species (P = 0.002). In pairwise tests the Indian Ocean species was classified correctly 100% and the central IWP species 90% of the time when compared with each other (Table 4). The four remaining lineages in dataset A (*L. cinerea* from NW Australia, NE Australia, Sulawesi and the Indian Ocean) could not be distinguished from each other and were poorly classified in the majority of cases based on shell-shape alone (Table 2).

The two cryptic *cinerea* species in the southwest Pacific were not significantly different from each other based on PC1 alone, but PC2 was highly significant (Table 3) as was MANOVA and Hotelling's *t* test on all three PCs (Table 1, 3). *Lunella* aff. *cinerea* from Fiji was correctly identified 93% of the time (86% when jackknifed) (and *L.* aff. *cinerea* SW Pacific from Vanuatu, American Samoa, Solomon Islands and New Caledonia was identified correctly 73% of the time (64% jackknifed).

Significant differences were observed among the species in the *Lunella coronata* West Indian Ocean subclade at PC2 (Table 3) as well as across all three PCs in MANOVA (Table 1). Discriminant analyses of EFDs showed that *L.* aff. *coronata* ophiolite was distinguished correctly in all cases (PP = 100%, jackknifed PP = 100%). *Lunella coronata* from the east coast of Africa extending along the Arabian Peninsula and into India (*L. coronata* coronate) was classified correctly 87% of the time (74% jackknifed; 96% of the time when compared only with the ophiolite morph, Table 4). The species from Oman was not classified correctly in the majority of cases (except when jackknifed, PP = 71%). Samples of this species from Masirah Island, Oman were similar to each other (and indeed to the -2 *S.D.* shell outline for PC1 in Figure 5C). However, one sample from Khor Rouri, Salalah, near the Yemen border of Oman was more similar to *L. coronata* coronate (see Fig. 6C). All misclassified specimens of *L.* aff. *coronata* Oman were wrongly classified as *L. coronata* coronate and in the case of the specimen from Salalah, with a high probability (PP = 93%).

Significant regressions were found between shell area (as a proxy for size) and shell shape, although contributions to total variation were low in most cases (corrected regression R^2 ranging from 5.2% for Dataset E to 42.5% for dataset A). Examination of the data showed that shell area contributed to 27% and 15.5% of the variation in PC1 and PC2 respectively for Dataset A, 28.4% to PC2 of Dataset B, 28.1% to PC2 of Dataset C, 6.7 % and 15.5% to PCs 1 and 2 respectively of Dataset D and 6.3% to PC1 of Dataset E (all corrected R^2). Although it did not correspond to a large proportion of the variation used to distinguish species, size was a useful character for separating several cryptic species (discussed below).

Identification of type species

Comparison of the holotype for *Lunella cinerea* and the figure of *L. versicolor* with all seven putative species in the

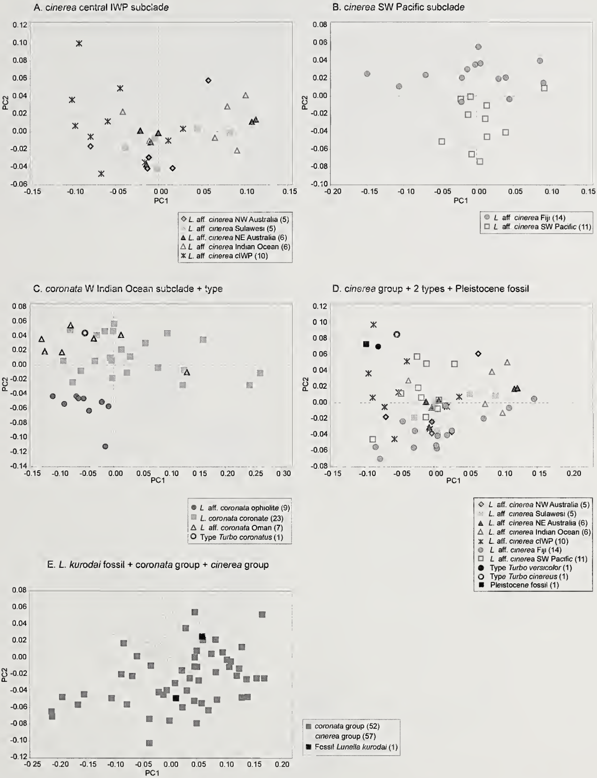

Figure 6. Plots of the first two principal components. Sample numbers in parentheses following species names. **A**, Dataset A: *cinerea* group - central IWP subclade: PCs explain 63% and 17% of variance, respectively. **B**, Dataset B: *cinerea* group - southwest Pacific subclade: PCs explain 56% and 22% of variance, respectively. **C**, Dataset C: *coronata* group - west Indian Ocean subclade including holotype of *Lunella coronata*: PCs explain 65% and 12% of variance, respectively. **D**, Dataset D: *cinerea* group including holotype of *L. cinerea*, figure of type specimen of *L. versicolor* and fossil: PCs explain 56% and 23% of variance, respectively. **E**, Dataset E: *cinerea* group, *coronata* group and *L. kurodai* fossil: PCs explain 61% and 12% of variance, respectively.

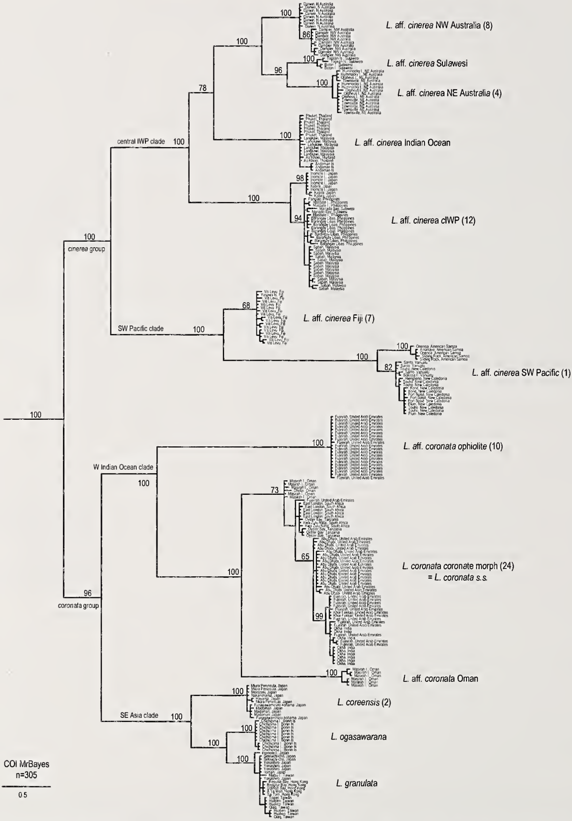
100
100
86
L. aff. cinerea NW Australia (8)
100
L. aff. cinerea Sulawesi
96
100
L. aff. cinerea NE Australia (4)
78
central IWP clade 100
100
L. aff. cinerea Indian Ocean
98
100
94
L. aff. cinerea cIWP (12)
100
cinerea group
68
L. aff. cinerea Fiji (7)
SW Pacific clade 100
100
100
82
L. aff. cinerea SW Pacific (1)
100
100
L. aff. coronata ophiolite (10)
W Indian Ocean clade
100
73
65
100
L. coronata coronate morph (24)
= L. coronata s.s.
99
96
coronata group
100
L. aff. coronata Oman
100
L. coreensis (2)
SE Asia clade 100
100
L. ogasawarana
100
100
L. granulata
COI MrBayes
n=305
0.5

Figure 7. Phylogeny based on Bayesian analysis of 305 *COI* sequences using MrBayes. Outgroup taxa have been trimmed for clarity and only the *cinerea* and *coronata* groups are shown. Branches with less than 50% support have been collapsed. Support for nodes is based on posterior probabilities (*PP*); values for intraspecific nodes are not shown except between clades recognized in Williams *et al.* (2011). Numbers after species names are number of new sequences added in this study.

cinerea group (dataset D) suggests that the former is most similar to *L.* aff. *cinerea* cIWP (Japan, Philippines, Sulawesi, Sabah; *PP* = 76%) and the latter to *L.* aff. *cinerea* SW Pacific (New Caledonia, American Samoa, Vanuatu, Solomon Islands; *PP* = 64%). However, *L.* aff. *cinerea* central IWP was misclassified 50% of the time and *L.* aff. *cinerea* cIWP 36%, so these conclusions are weak and we are unable to assign either type with confidence to any species.

Comparison of the holotype of *Lunella coronata* with specimens from three West Indian Ocean species, suggests that it is most similar to *L.* aff. *coronata* Oman (*PP* = 62%), although with weak support. It was very rarely identified as the ophiolite morph (<1%).

Fossils

Two complete fossil specimens of *Lunella kurodai* in the NHMUK paleontology invertebrate collection (registration: NHMUK PI TG 26505-07) were used in an analysis of outline shape with all samples for 13 species from the *cinerea* and *coronata* groups (dataset E). We analyzed this dataset first by species then by species complex. One fossil specimen was most closely associated with *L. granulata* (*PP* = 76%) and the other with *L. cinerea* from Fiji (*PP* = 47%), although both associations were weak. Combining species into species groups and re-analyzing the data show that first specimen strongly associates with the *coronata* group (*PP* = 99%) but the other weakly with the *cinerea* group (*PP* = 75%).

A complete Pleistocene specimen from Java belonging to the *cinerea* group (Sonde, Central Java; Leiden; Rijksmuseum van Natuurlijke Histoire (Leiden, Netherlands): RMNH 11545) is most similar to *Lunella* aff. *cinerea* cIWP although with weak support (*PP* = 74%). One of the assumptions, homogeneity of variances is violated in the first two PCs in this dataset (Dataset E), so although minor deviations are not important (StatSoft online manual) results for this dataset should be viewed with caution.

DISCUSSION

Utility of EFA for distinguishing cryptic species

Outline analyses clearly distinguished one species, which was correctly classified in all cases. *Lunella* aff. *coronata* ophiolite was found to differ significantly in outline from both *L. coronata* coronate and from *L.* aff. *coronata* Oman, and was classified correctly when compared with these species separately or together. The ophiolite species tends to be low spired and is wider than it is high, whereas the coronate species has a higher spire, with a more inflated body-whorl and an anterior flange-like extension of the columella (Fig. 2). The ophiolite species is also smaller and is less coronate than *L. coronata* coronate, with coronations weak or absent from the last part of the body whorl (Fig.2). The highest spired shells in *L. coronata* coronate tend to occur in the Persian Gulf (ESU O' in Williams *et al.* 2011). The Oman species and the coronate species could not be distinguished from each other by shell shape.

Lunella aff. *cinerea* cIWP was found to differ significantly in outline from the Indian Ocean species in pairwise tests. The central IWP species has an anterior flange-like extension of the columella (most developed in Japanese specimens), which is slightly retroflected when viewed from below (Fig. 2). The low spire and developed anterior flange on the columella is characteristic of juveniles of all *Lunella* species, but is retained in some species. The Indian Ocean species, in contrast, are high-spired (for *Lunella*) and do not tend to have an anterior flange-like extension of the columella (Fig. 2).

The Fijian endemic, *Lunella* aff. *cinerea* Fiji, was also significantly different from its sister species, *L.* aff. *cinerea* SW Pacific, in pairwise tests. It has a higher spire and a less inflated body whorl and has more pronounced spiral ribs (Fig. 2). The more widely distributed SW Pacific species has a fig-like shape with an anterior flange-like extension of the columella and a low spire. It is mostly smooth or only weakly ribbed and sometimes has a polished appearance. Specimens of *L.* aff. *cinerea* SW Pacific can grow to a very large size (height > 4.5 cm) whereas shells of *L.* aff. *cinerea* from Fiji tend to be smaller (height < 2.5 cm).

These results indicate that morphometric analysis of shell shape is a useful method for distinguishing some cryptic taxa in *Lunella*. Visualization of variation in shell outline also helps to highlight features of the shells that can be used to identify taxa. However on its own, shell shape and size were not enough to separate all species in this study. Larger sample sizes would likely increase the power of analyses, but it seems doubtful that even with much larger sample sizes that all *Lunella* species could be distinguished purely by their shell outline. Outline analyses have, however, been used successfully to distinguish cryptic species in other mollusk taxa (Puillandre *et al.* 2009, 2010, de Aranzamendi *et al.* 2010, Costa *et al.* 2010, Van Bocxlaer and Schultheiß 2010, González-Wevar *et al.* 2011). EFA in combination with quantitative genetic analysis has also been shown to detect evidence of selection acting on biological shapes (*e.g.* Polihronakis 2008). Outline analyses are particularly useful in taxa where homology of landmarks is uncertain (Van Bocxlaer and Schultheiß 2010).

In the case of *Lunella*, additional characters, such as shell sculpture and color of opercula are required to distinguish most species. Soft tissue characters are also useful (see also Meyer *et al.* 2005), although not discussed here. Even using these additional characters it is difficult to distinguish three species (*L.* aff. *cinerea* NW Australia, *L.* aff. *cinerea* NE Australia and *L.* aff. *cinerea* Sulawesi). If it is not possible to

Table 1. Eigenvalues and contributions of the principal components that describe 95% of the variation based on normalized elliptic Fourier descriptors of shell shape. Datasets A–C are concerned with determining whether cryptic species can be recognized; datasets C–E are concerned with identifying unknown specimens (fossils or types). *F* tests are for values of Wilk's λ from MANOVA tests using species as groups and their associated probabilities (*P*). Values that are significant after Sequential Bonferroni are marked with an asterisk.

Dataset/Principal Component	Eigenvalues x 10^{-3}	Cumulative Proportion (%)	*F*	*p*
A. *cinerea* group - central IWP subclade			2.40	0.012*
1	3.96	63.0		
2	1.06	79.9		
3	0.42	86.6		
4	0.30	91.3		
5	0.15	93.6		
6	0.12	95.5		
Total variance	6.28			
B. *cinerea* group - SW Pacific subclade			11.80	< 0.001*
1	3.07	56.0		
2	1.19	77.7		
3	0.48	86.4		
4	0.22	90.4		
5	0.13	92.8		
6	0.09	94.5		
7	0.09	96.0		
Total variance	5.48			
C. *coronata* group - W Indian Ocean subclade + type			11.80	< 0.001*
1	8.44	64.3		
2	1.60	76.4		
3	1.12	84.9		
4	0.49	88.7		
5	0.29	90.9		
6	0.23	92.7		
7	0.19	94.1		
8	0.15	95.2		
Total variance	13.13			
D. *cinerea* group + 2 types + Pleistocene fossil			3.20	< 0.001*
1	3.60	56.0		
2	1.45	78.7		
3	0.44	85.5		
4	0.26	89.6		
5	0.16	92.1		
6	0.10	93.6		
7	0.09	95.0		
Total variance	6.42			
E. *cinerea* group + *coronata* group + fossil *L. kurodai*			9.69	< 0.001*
1	6.71	60.8		
2	1.34	73.0		
3	1.30	84.7		
4	0.37	88.1		
5	0.24	90.3		
6	0.20	92.1		
7	0.19	93.8		
8	0.12	94.9		
9	0.11	95.9		
Total variance	11.03			

Table 2. Univariate analyses for datasets A–C. F tests are for values of Wilk's λ and their associated probabilities (*P*). Values that are significant after Sequential Bonferroni correction are marked with an asterisk.

Dataset	PC	*F*	*P*
A. *cinerea* group - central IWP subclade	1	4.20	0.009
	2	0.83	0.52
	3	1.51	0.23
B. *cinerea* group - SW Pacific subclade	1	0.34	0.56
	2	35.80	< 0.001*
	3	0.10	0.76
C. *coronata* group - W Indian Ocean subclade + type	1	4.67	0.016
	2	30.43	< 0.001*
	3	1.31	0.28

identify species morphologically, like other authors (*e.g.* Collin 2005, Puillandre *et al.* 2010), we believe that they should still be recognized as distinct entities and given specific status, if genetic divergence is sufficiently high. The problem of determining cut off levels for genetic divergence is ongoing, although new statistical methods that determine the transition between coalescent and speciation branching patterns may be helpful (*e.g.* Pons *et al.* 2006; although see Williams *et al.* 2011 for comments about using this method for low dispersal species).

Identification of type species

The holotype of *Lunella coronata* was figured by Martini and Chemnitz (1781: pl. 180, figs 1791–1792) and photographed by Cernohorsky (1974). The type locality is given by Gmelin (1791) as "streto Malaccensi" (Strait of Malacca), however the specimen in the Copenhagen Museum is labeled as coming from Nicobars (the Nicobar Islands). We have no records of *coronata* species from either locality, so we assume these localities are erroneous. Morphometric analysis of the holotype of *L. coronata* did not show strong association with either *L.* aff. *coronata* Oman or *L. coronata* coronate however it was only very rarely identified as the ophiolite morph. Although analysis of shell outline excludes only *L.* aff. *coronata* ophiolite, other characters are useful. The Oman species is umbilicate, whereas examination of the holotype shows that the specimen is imperforate (see shell in Fig. 3). The distinctly coronate spines on the holotype, large shell size and general shape confirm that it is not *L.* aff. *coronata* ophiolite. The only other likely member of this clade, *L. viridicallus*, is distinguished by its green columella (Alf and Kreipl 2003), a character not present in the type. Thus we are confident that *L. coronata* coronate, which is both imperforate and coronate, is in fact *L. coronata s.s.*

EFA analyses were not sufficient to identify which species best corresponds to *Lunella cinerea s.s.*, although some species could be definitively excluded. The most similar species was *L.* aff. *cinerea* cIWP, but statistical support was weak. Unfortunately the original description has no type locality and no other shell characters have been identified that might be used to distinguish between species, so we are unable to assign the holotype with confidence. The slight damage to the aperture may also have affected analyses.

It was of particularly interest to accurately determine which species corresponds to *Lunella versicolor*, as this is the type species of the genus. In the recent past this species has been thought to be synonymous with *L. cinerea sensu lato* (equivalent to the *cinerea* group species complex). Unfortunately, although outline morphometric analyses suggest that the figure Gmelin refers to in Martini and Chemnitz (1781) most resembles *L.* aff. *cinerea* SW Pacific, the posterior probability was weak. It would be useful to repeat these analyses if the type specimen could be located, as the figure may not have been representative.

Fossils

Two complete specimens of *Lunella kurodai* from late Early Miocene in Japan were used in an analysis of outline shape with 13 species from the *cinerea* and *coronata* groups. One specimen associated strongly with the *coronata* group and with *L. granulata* from China and Vietnam in particular. The other associated weakly with the *cinerea* group and very weakly with *L.* aff. *cinerea* Fiji. Given that the fossils were found in Japan it seems likely that the first specimen is more representative. Indeed, previous workers have suggested that *L. kurodai* is most similar to *L. coreensis* (Itoigawa, 1955), which occurs on coasts of mainland Japan. Fortunately additional shell characters can be used to place this fossil more definitively within the phylogeny. It has spiral ribs of small granules, a characteristic found only in the *coronata* group, suggesting that it is a member of this group.

A complete Pleistocene specimen from Java belonging to the *cinerea* group falls close to the range of *Lunella* aff. *cinerea* cIWP and is most often classified as that species. The fossil is sufficiently recent to be likely to represent an extant species, however, this locality has not been included in genetic analyses so its identification as *L.* aff. *cinerea* cIWP is provisional. Another caveat to consider is that the holotype for *L. cinerea* was also quite similar to this fossil specimen. If there were another cryptic lineage in West Indonesia, it may prove a better match to the holotype than the central IWP lineage.

Morphological divergence in allopatric versus sympatric species

This study highlights that morphological differences are strongest in the *coronata* group, where species ranges

Table 3. The proportion of specimens for three datasets with cryptic species that could be accurately classified *post hoc* based on canonical variate analysis. Numbers in parentheses are jackknifed values.

A. *cinerea* central IWP subclade

		***Post hoc* classification**				
True classification	**% specimens identified correctly**	NW Australia	Sulawesi	NE Australia	Indian Ocean	cIWP
L. aff. *cinerea* NW Australia	20 (20)	1 (1)	2 (2)	0 (0)	1 (1)	1 (1)
L. aff. *cinerea* Sulawesi	40 (20)	0 (1)	2 (1)	1 (1)	1 (1)	1 (1)
L. aff. *cinerea* NE Australia	16.7 (0)	0 (0)	3 (3)	1 (0)	1 (2)	1 (1)
L. aff. *cinerea* Indian Ocean	66.7 (33.3)	1 (1)	0 (2)	1 (1)	4 (2)	0 (0)
L. aff. *cinerea* cIWP	100 (70)	0 (2)	0 (0)	0 (1)	0 (0)	10 (7)
Total	56.2 (34.4)	2 (5)	7 (8)	3 (3)	7 (6)	13 (10)

B. *cinerea* SW Pacific subclade

		***Post hoc* classification**	
True classification	**% specimens identified correctly**	Fiji	SW Pacific
L. aff. *cinerea* Fiji	92.9 (85.7)	13 (12)	1 (2)
L. aff. *cinerea* SW Pacific	72.7 (63.6)	3 (4)	8 (7)
Total	84.0 (76)	16 (16)	9 (9)

C. *coronata* W Indian Ocean subclade

		***Post hoc* classification**		
True classification	**% specimens identified correctly**	ophiololite	coronate	Oman
L. aff. *coronata* ophiolite	100 (100)	9 (9)	0 (0)	0 (0)
L. coronata coronate	87.0 (73.9)	1 (1)	20 (17)	2 (5)
L. aff. *coronata* Oman	28.6 (71.4)	0 (0)	5 (2)	2 (5)
Total	79.5	10	25 (19)	4 (10)

Table 4. Percentage of correct classifications in pairwise discriminant analyses of species and total percentage of cases with correct classification. Numbers in parentheses are results after jackknifing. Hotelling's *t2* test (*H t2*) after jackknifing and associated (*F*) and probability (*P*). Values that are significant after Sequential Bonferroni correction are marked with an asterisk.

Dataset A	% Correct Classifications								
cinerea group	NW Australia	Sulawesi	NE Australia	Indian Ocean	cIWP	Total	*H t2*	*F*	*p*
NW Australia vs. Sulawesi	60% (40%)	60% (60%)	–	–	–	60% (50%)	0.067	0.02	0.997
NW Australia vs. NE Australia	60% (80%)	–	50% (33%)	–	–	64% (55%)	0.28	0.07	0.974
NW Australia vs. Indian Ocean	80% (80%)	–	–	67% (67%)	–	73% (73%)	2.80	0.70	0.585
NW Australia vs. cIWP	80% (80%)	–	–	–	90% (90%)	87% (87%)	21.85	6.07	0.013
Sulawesi vs. NE Australia	–	40% (40%)	67% (67%)	–	–	55% (55%)	1.14	0.28	0.836
Sulawesi vs. Indian Ocean	–	60% (60%)	–	67% (83%)	–	64% (73%)	4.25	1.06	0.432
Sulawesi vs. cIWP	–	80% (80%)	–	–	100% (90%)	93% (87%)	9.59	2.66	0.105
NE Australia vs. Indian Ocean	–	–	80% (67%)	67% (67%)	–	67% (67%)	4.02	1.04	0.431
NE Australia vs. cIWP	–	–	83% (83%)	–	80% (80%)	81% (81%)	16.87	4.76	0.023
Indian Ocean vs. cIWP	–	–	–	100% (100%)	100% (90%)	100% (94%)	36.68	10.35	0.002*

Dataset B	% Correct Classifications					
cinerea group	Fiji	LM	Total	*H t2*	*F*	*p*
Fiji vs. SW Pacific	93% (93%)	82% (73%)	88% (84%)	35.76	10.84	< 0.001*

DATASET C	% Correct Classifications						
coronata group	ophiolite	coronate	Oman	Total	*H t2*	*F*	*p*
ophiolite vs. coronate	100% (100%)	96% (96%)	–	97% (97%)	66.87	20.75	<0.001*
ophiolite vs. Oman	100% (100%)	–	100% (100%)	100% (100%)	78.09	22.02	<0.001*
coronate vs. Oman	–	78% (78%)	71% (71%)	77% (77%)	7.05	2.18	0.116

overlap. In the *cinerea* group, where species occur in complete allopatry (based on sampling to date), shell morphology is more similar and it is more difficult to distinguish species than in the *coronata* group. This is particularly relevant since all species in the *cinerea* group share very similar habitats, occurring in the intertidal area on rocky shore beaches. As such, it appears likely that the *cinerea* group is another example of non-adaptive radiation (Gittenberger 1991), where genetic diversification has not resulted in strong phenotypic differences or adaptation to new niches. These findings suggest that, as in many other taxa (*e.g.* Adams *et al.* 2007, Scott *et al.* 2010), interspecific competition in *Lunella* may play an important role in phenotypic diversification in this group. Allopatric ranges in the *cinerea* group mean that species are unlikely to come into contact as adults, minimizing selection between sister species. Sympatry and phenotypic differences between at least some *coronata* group species may have come about separately after species came back into contact secondarily after originating allopatrically or as a result of ecological speciation (*e.g.* Rundell and Price, 2009).

It also has some bearing on estimates of marine diversity in the IWP, particularly with respect to low dispersal invertebrates. If morphological divergence is driven most strongly in sympatry, we might predict that cryptic species will occur most often in taxa with allopatric speciation. Allopatric species complexes may be most common in the central IWP area where shallow coastal habitat is distributed over two dimensions. Species co-occurring along coastlines with continuously distributed habitat may show greater morphological divergence and so cryptic species are less likely. Therefore, taxonomic and biogeographic studies could benefit from focusing on low dispersal species in the central IWP.

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Biogeographical and ecological determinants of land snail diversification on islands*

Christine E. Parent

Section of Integrative Biology, University of Texas at Austin, 1 University Station C0930, Austin, Texas 78712, U.S.A.
Correspondence, Christine E. Parent: ceparent@gmail.com

Abstract: The equilibrium theory of island biogeography as originally proposed by R. MacArthur and E. O. Wilson relied on the ecological processes of colonization and extinction to determine the species diversity of islands. Although they were well aware of the potential contribution of speciation within-island to species diversity, MacArthur and Wilson's equilibrium model was purely ecological and did not incorporate the evolutionary process of diversification. This might seem surprising given that the best-known examples of adaptive radiation, the diversification of a single ancestral species into descendant species occupying a wide variety of ecological niches, are found on island systems. Recently, it has become possible to quantify the relative contribution of between-island colonization and within-island speciation to species diversity with the use of molecular phylogenies. By providing an estimate of the historical relationship among species, phylogenies can be used in combination with information on species' geographical distributions to infer the geographical zone of origin of species. Here I use this process-oriented analysis to determine the main predictors of diversification in three unique insular land snail lineages: the Galápagos Bulimulidae, the Hawaiian Succineidae and the Hawaiian Achatinellinae. I find that in Galápagos bulimulid land snails, species richness resulting from between-island colonization is mainly driven by island area and insularity, whereas species richness resulting from within-island speciation is mainly determined by habitat heterogeneity. In contrast, I find that within-island species diversification is driven by island area in Hawaiian succineids, and the probability of speciation via between-island colonization in this group is significantly greater on younger islands. None of best multiple regression models proposed to explain variation in Hawaiian Achatinellinae species diversity were significant.

Key words: Achatinellinae, Bulimulidae, Succineidae, Galápagos, Hawaii

For years biologists have recognized the unique value of oceanic islands for the study of ecological and evolutionary processes. If part of an archipelago, islands become a series of replicates within a large evolutionary natural experiment, making them scientifically even more appealing. Over the years, research focusing on island biotas has led to the development of ideas within a wide range of fields including biogeography and the formation of species.

The theory of island biogeography as initially proposed by MacArthur and Wilson (1963, 1967) stated that variation in rates of inter-island colonization and extinction generates and maintains island species diversity. Less isolated islands presumably receive a high number of colonists and, therefore, reach higher equilibrium diversity. Larger islands carry species with larger population size, and because larger populations are less likely to be driven to extinction by stochastic processes, larger islands are expected to maintain higher equilibrium diversity. Larger islands often also have greater habitat heterogeneity, which in turn could allow for the coexistence of more species diversity. However, because island area and habitat heterogeneity are frequently correlated, it has been difficult to disentangle the roles of habitat heterogeneity and island area. Since the original formulation of the theory of island biogeography, numerous studies have shown that island insularity and area (and sometimes habitat heterogeneity) are significant predictors of total island species richness (Triantis *et al.* 2008).

Though MacArthur and Wilson's ecological theory of island biogeography includes the process of speciation (MacArthur and Wilson 1963, 1967), the empirical tests of their model most often assumed that the only source of species diversity is colonization from external pools. However islands are also known as natural laboratories of evolution, and many of the most famous cases of island diversity are the result of within-island formation of new diversity via speciation (Losos and Ricklefs 2009 and references therein). Until recently it has been impossible to determine the relative contribution of speciation due to between-island colonization and within-island speciation to total island species richness, and therefore impossible to disentangle the effects that biogeographical factors (such as island area) have on colonization versus speciation. However, the increasing accumulation of molecular phylogenies has prompted a new approach to the species-richness problem with the integration of evolutionary history in community assembly studies. Here, phylogenies can be used in combination with geographical distributions and simple parsimony to infer the geographical zone of origin of species. In insular contexts, this implies that the processes of within-island speciation versus speciation due to between-island colonization can be differentiated, quantified, and studied separately (Fig. 1). By partitioning species diversity into these two components, it becomes possible to separately evaluate the importance of biogeographical factors (such as island insularity, area, habitat heterogeneity, and age) on within-island speciation and between-island colonization (Parent and Crespi 2006). This is a novel, conceptually simple approach to a very old and general problem about how environmental variation (here, species richness) arises. This approach should inform us of the ecological and evolutionary processes involved in the formation of species at the community level, as well as the relative importance that various biogeographical and ecological factors have on these processes.

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Many organisms have diversified within archipelagos, and, in general, invertebrate lineages have evolved into great numbers of species. However, land snails are of particular interest: with their low dispersal ability over short temporal and spatial scales, combined with their potential for long distance dispersal over longer time scales, they are ideal candidates for diversification on islands. Not only can land snails reach very isolated islands, once established they will usually diversify and multiply, presumably as a result of the combination of geographical isolation and local adaptation.

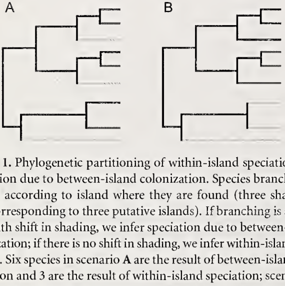

Figure 1. Phylogenetic partitioning of within-island speciation and speciation due to between-island colonization. Species branches are shaded according to island where they are found (three shades of gray corresponding to three putative islands). If branching is associated with shift in shading, we infer speciation due to between-island colonization; if there is no shift in shading, we infer within-island speciation. Six species in scenario **A** are the result of between-island colonization and 3 are the result of within-island speciation; scenario **B** depicts 3 speciation events due to between-island colonization and 6 following within-island speciation events.

In this paper, I first outline a methodological approach combining phylogenetic and biogeographical data to partition species richness on islands into its driving processes. I then use this approach to evaluate the importance of biogeographical and ecological factors in generating species richness on islands via speciation *in situ* and speciation due to between-island colonization in three endemic lineages of insular land snails: the bulimulid land snails of the Galápagos, and the amber snails (Succineidae) and tree snails (Achatinellinae) of the Hawaiian Islands. I conclude the paper with a discussion of the prospects and limitations of the approach to the study of island diversity presented here.

STUDY SYSTEMS

Galápagos Islands

The Galápagos Islands are located in the Pacific Ocean, about 960 km west of the South American coast, straddling the equator at the 90th meridian west. The Galápagos archipelago is composed of 13 major islands larger than 10 km^2, and numerous smaller islands, islets and rocks, for a total of about 8000 km^2 of land spread over 45 000 km^2 of water (Snell *et al.* 1996). Of these islands, Isabela is the largest (more than the total area of all the other islands combined) with an area of 4588 km^2 and the highest, with a maximum elevation of just over 1700 m. Isabela is formed by six volcanoes interconnected mostly by barren lava flows, that may represent a geographical barrier to dispersal analogous to open water for land snails.

The Galápagos Islands are a young, oceanic, volcanic archipelago with a relatively well-understood geological history (Nordlie 1973, Swanson *et al.* 1974, Bailey 1976, Cox 1983, Hall 1983, White *et al.* 1993, Geist 1996). Potassium-argon (K-Ar) age determinations and marine fossils indicate a maximum age of the oldest currently existing island on the order of 3 million years (Myr) ago, whereas geological plate motion models set a maximum age of emergence around 4 Myr ago, depending on the velocity of the Nazca plate (White et al. 1993, Geist 1996). In contrast to the roughly linear arrangement by age of the Hawaiian Islands (Price and Clague 2002), the Galápagos Islands are clustered into groups of similar age (White *et al.* 1993). Vegetation on Galápagos can be separated into six (or seven) altitudinal zones (Wiggins and Porter 1971, van der Werff 1979) and the plant species composition of each zone is a reflection of the humidity level of the zone, with moisture level increasing with elevation (McMullen 1999).

Like that of many other isolated oceanic islands, the Galápagos fauna is impoverished and taxonomically unbalanced when compared to Neotropical source areas. Because of their isolation and arid climate, the Galápagos Islands have been successfully colonized by only a subset of the diverse flora and fauna of the closest continent. Although relatively few lineages of Galápagos terrestrial fauna have diversified within the archipelago (associated with adaptation or not), the species resulting from this diversification process constitute a large proportion of the terrestrial fauna of the islands.

Galápagos Bulimulidae

With 71 described species grouped in one genus, the radiation of Galápagos bulimulid land snails is the most spectacular in the biota of these islands in terms of species number (Chambers 1991, Parent *et al.* 2008). Of the nine other land snail genera that have colonized the islands, only one radiated and gave rise to 4 species (Smith 1966). All Galápagos bulimulid species are endemic, and current phylogenetic evidence based on multiple independent molecular markers (mitochondrial and nuclear DNA genes, as well as microsatellite markers) suggests that all species studied in detail are single island endemics (Parent and Crespi 2006, Parent 2008).

Bulimulids have colonized all of the major Galápagos Islands, and they are found at all elevations except on shorelines composed mainly of sandy beaches and lava rocks. Many species have limited and patchy distribution range, most often restricted to a single vegetation zone. Past records indicate that up to 11 species could be found at a single location (Coppois 1985), but finding four or five coexisting species in a given habitat is more common. Galápagos bulimulid species vary remarkably in shell morphology (size, shape, color, and color pattern), and this phenotypic variation has been found to be associated with various aspects of ecological variation, including vegetation zones, related moisture levels, and microhabitat (Coppois and Glowacki 1983, Parent 2008). Furthermore, a significant positive correlation between shell shape (degree of shell roundness) and elevation suggests that snail species have adapted morphologically to the varying moisture levels (Parent 2008). Since plants provide food and shelter, and probably most importantly, habitat structure, land snails can potentially adapt to different plant species for feeding, hiding, or resting.

Parent and Crespi (2006) found a combination of within and between-island speciation in Galápagos bulimulid snails. Polyphyletic land snail assemblages were found on islands centrally located in space and time (Isabela, Santa Cruz, Pinzón, Santiago), whereas the more isolated (again in both space and time) islands of Española, San Cristobal and Floreana were mostly occupied by monophyletic assemblages resulting from within-island diversification.

Hawaiian Islands

The Hawaiian islands are an archipelago of eight major islands, several atolls, numerous smaller islets, and seamounts in the Pacific Ocean, located about 3,000 km from the nearest continent. The islands were formed as the Pacific plate moved northwestward over a stationary "hot spot" in the earth's mantle. Generally the islands are divided into two categories: the young and high islands versus the old and lower islands. Currently, the oldest island is Kure Atoll (29 Ma) and the oldest high island is Kauai (5.1 Ma), whereas the youngest island, Hawaii, is less than 0.6 Ma and still in volcanically active. For the case studies presented in this paper I have restricted the analyses to biotas found on the young and high islands.

Similarly to Galápagos Islands, the Hawaiian archipelago is the host of unique organisms that are the products of evolution in isolation over tens of millions of years. The islands' extreme isolation, diversity of habitats, and dynamic geology have given rise to remarkable organismal diversity (Ziegler 2002). Over 750 described non introduced land snail species are found in Hawaii, and over 99% of these species are unique (endemic) to the islands (Cowie 1995, Cowie *et al.* 1995).

Below I test for the effect of island area, elevation, habitat diversity (measured as the total number of plant species), isolation, and age on total island species diversity, species diversity resulting from within-island speciation and species diversity resulting from between-island colonization. Similarly to the Galápagos Islands data set, I first tested for pairwise correlations among the independent variables, and then determined tolerance levels for the pair of independent variables that were significantly correlated. The only significant correlation was found between-island area and elevation ($r = 0.893$, $t = 4.434$, $P = 0.007$), so that larger islands are also higher. Tolerance value for elevation regressed on area (Tolerance = $1\text{-}R^2 = 0.202$) is too low for both variables to be included in the same model (Quinn and Keough 2002), and therefore I excluded island elevation from all further analyses on the Hawaiian data sets.

Hawaiian Succineidae

There are 42 recognized Hawaiian succineid species. All Hawaiian succineids are endemic, and 35 of them are thought to be single-island endemics (Cowie *et al.* 1995). Initially the Hawaiian succineids were thought to be monophyletic (Zimmerman 1948), but more recent molecular work suggests that this group might in fact be the result of multiple colonization events Rundell *et al.* (2004). However this does not affect the inference of the mode of diversification as presented in this paper; island clades that are monophyletic are still inferred to be the result of within-island speciation, whereas species that have their closest relative on another island or somewhere outside the archipelago are considered speciation events due to colonization. The Hawaiian Succineidae have radiated into a diverse array of habitats, from the very dry coasts to highland rainforests (Cowie 1995, Cowie *et al.* 1995). Holland and Cowie (2009) noted that there is an association between shell morphological variation and ecological variation suggesting that diversification within this group might be at least partly adaptive. If this is the case, I expect island habitat diversity to be a significant contributor of species richness in this group, particularly when considering species resulting from within-island speciation.

Holland and Cowie (2009) found weak support for the progression rule of successive colonization from older to younger islands. However, the highest diversity in this group of land snails is clearly not found on the older islands: the island of Hawaii is less than 0.6 Ma old, and harbors 22 of the 42 recognized species (Cowie *et al.* 1995), suggesting that increasing island age might not be associated with greater species diversity.

Hawaiian Achatinellinae

With their remarkable phenotypic diversity, for years the Hawaiian tree snails (Achatinellinae) have fascinated professional and amateur biologists alike. There are 99 recognized species in the group distributed in 4 genera and all are considered to be single-island endemics (Cowie *et al.* 1995, Holland and Hadfield 2004). Presumably the diversification in this group is not adaptive but rather is associated with geographical isolation. Most species are now endangered or extinct, and the phylogenetic relationship among the species in this group is therefore inferred based on a very limited number of species. I used the molecular phylogeny presented in Holland and Cowie (2009), which was first published by Holland and Hadfield (2004) to quantify the processes of diversification in this group.

Biogeographical and ecological correlates of diversification

The extent of within-island and between-island speciation in any taxonomic group with multi-island distribution on an archipelago can be determined by combining information about the geography and phylogeny of the species. For islands with at least one species, we can infer at least one speciation event due to inter-island colonization (phylogenetic information is not needed). For islands with more than one species, the existence of distantly related species on the same island suggests speciation as a result of colonization (Fig. 1). On the contrary, *in situ* speciation is indicated by a clade of sister species on an island where a clade originated by the colonization of a single species that diversified into multiple species thereafter (Fig. 1). The above inferences are the most parsimonious explanations for the observed patterns given the data at hand, and do not necessarily correspond to what happened. For example, mitochondrial (mt) DNA introgression could potentially lead to the erroneous inference of within-island speciation (Losos 2011). Indeed, if colonizing individuals happen to interbreed with individuals of an already established population of another species, their descendants' mtDNA could leave a signature of within-island speciation on the phylogeny. This particular type of erroneous inference can be avoided if the phylogeny used to infer speciation processes is based on multiple independent genetic markers (as opposed to exclusively rely on mtDNA). Another possibility is that multiple colonization events coupled with extinction of the ancestral species on the source island would leave the erroneous inference of within-island speciation on the colonized island. Although this scenario of double colonization followed by extinction is certainly a possibility, it seems unlikely to account for large groups of species since it would require several extinction events (Losos and Parent 2009). Unfortunately, the latter erroneous inference cannot be avoided by adding molecular data, and unless there is a good fossil record for the studied species (which is rarely the case for species occurring on volcanic oceanic islands), it is impossible to empirically rule out the double-colonization followed by extinction scenario.

Once the extent of within-island vs. between-island diversification is quantified, it becomes possible to test specific predictions related to the relative importance of biogeographical factors on the processes of diversification (Table 1). Large island area is considered an important factor promoting speciation *in situ* by providing more opportunity for isolation within an island, so that populations that become geographically isolated have the potential to diverge and split into different species. The greater habitat heterogeneity of larger islands can also affect the rate of within-island speciation by providing additional opportunity for diversification through what is referred to as ecological speciation (*e.g.*, Rundle and Nosil 2005, Funk *et al.* 2006), or as a result of greater persistence (lower extinction rates) of species that can escape the burden of competition by adapting to unoccupied habitats. In addition to area and insularity, island age can affect the total island species richness since older islands have had more time to accumulate species through colonization and speciation. In the colonization-extinction equilibrium context, age should only have a short-lived effect, with colonists filling up ecological space as it becomes available on islands. However, species diversity resulting from the presumably slower process of within-island speciation might be affected by island age, with older islands having more time to accumulate habitat diversity, and in turn more time to accumulate more species via this evolutionary process. Finally, the species diversity equilibrium is dynamic and changes through time because islands themselves change (Whittaker *et al.* 2008). Island age can therefore have indirect effects on oceanic island species richness by its effects on island area and elevation (volcanic islands shrink and sink over time), and island insularity (e.g., the geographical configuration of the archipelago is likely to change over time). Table 1 is a

Table 1. Predictors of land snail species richness, and the description of their predicted direct and indirect effects. The process(es) of species formation or maintenance each predictor is affecting is indicated in parentheses (S = within-island speciation; C = inter-island colonization; E = extinction), along with the direction of the predicted relationship with an increase in the value of the predictor (+ for positive; - for negative). § This effect is likely to become less significant as niches on islands are filled by colonists and newly formed species. Modified from Parent and Crespi 2006.

	Description	
Species Richness Predictor	Direct Effect(s)	Indirect Effect(s)
Island Area	- larger target for colonists (C+) - increased opportunities for isolation by distance (S+) - reduced extinction risk by potentially sustaining larger population size (E-)	- increased habitat diversity (S+, C+)
Island Elevation	- better target for colonists (C+)	- increased habitat diversity (S+, C+)
Island Habitat Diversity	- increased potential for adaptation (S+) - increased probability that colonists will find suitable habitat (C+)	none
Island Age	- more time to be colonized (C+)§ - more time to speciate (S+)§ - more time to go extinct (E+)	- increased (S+, C+) and followed by decreased (S-, C-) habitat diversity as island decreases in area - decreased island elevation and area due to erosion and subsidence (S-, C-, E+)
Island Isolation	- target harder to reach for colonists (C-)	- decreased habitat diversity because of lower colonization rate by plants (S-, C-)

comprehensive list of the potential direct and indirect effects different biogeographical and ecological factors might have on the processes of within-island speciation, speciation due to between-island colonization, and extinction.

RESULTS

Galápagos Bulimulidae

I re-analyzed data from Parent and Crespi (2006) to determine the importance of island area, habitat diversity (measured as the number of native plant species), island insularity (measured as distance from the nearest older major island), and island age, on island species diversity. Because collinearity between independent variables might confound the analyses I checked for redundancy by investigating tolerance levels for the independent variables. I first tested for pairwise correlations among the independent variables, and then determined tolerance levels for the pair of independent variables that were significantly correlated. The significant correlations were found between-island area and elevation (correlation coefficient $r = 0.690$, $t = 3.302$, $P = 0.006$), island area and habitat diversity ($r = 0.765$, $t = 4.114$, $P = 0.001$), island area and isolation ($r = 0.741$, $t = 3.824$, $P = 0.002$), and island elevation and age ($r = -0.638$, $t = -2.874$, $P = 0.014$). Tolerance values for all of these pairwise regressions ($\text{Tolerance} = 1\text{-}R^2 > 0.45$) were adequately high (Quinn and Keough 2002), and all variables could therefore be included in the regression models.

I used a phylogeny based on multiple independent DNA markers (updated from Parent and Crespi 2006) to distinguish species that arose *in situ* on an island from those that arrived by colonization from another island. In these analyses, I also included species that are the sole inhabitant of islands, because even if they are not represented on the phylogeny, they can safely be inferred as being the result of between-island colonization. Following this method I inferred 25 colonization and 15 speciation events for a total of 40 species distributed over 14 islands.

The best multiple regression model explaining the total bulimulid species richness among Galápagos islands includes island habitat heterogeneity and age (Table 2). Therefore, a richer fauna of bulimulid land snails is found on older islands harboring a more diverse range of habitats. When considering species richness resulting only from speciation due to between-island colonization, the best model includes island area and isolation. Larger islands presumably represent easier to reach targets for potential colonists. Moreover, larger islands have greater habitat diversity, and there is therefore a greater chance that a dispersing land snail will find a suitable habitat and establish on larger islands. The habitat diversity on islands is the only significant predictor of species richness due to *in situ* speciation (Table 2). This positive relationship between habitat and species diversity suggests that diversification

Table 2. Results of multiple regression analyses. The sample size (*N*) is provided for each model, as well as the standardized regression coefficient (β), the standard error for the regression standardized coefficient (*S.E.* of β) and the test statistic (t) for each independent variable entered in each model. *P* values for adjusted R^2 and β values are indicated as follow: † $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Snail group	Dependent variable	*N*	Independent variables	β	*S.E.* of β	t
Galápagos	Total species richness	26	island habitat diversity***	0.539	0.122	4.423
Bulimulidae	Adjusted $R^2 = 0.433$**		island age	0.111	0.081	1.364
	Between-island speciation	14	island area**	0.173	0.0425	4.07
	Adjusted $R^2 = 0.543$**		island isolation**	-0.00000621	0.0000017	-3.662
	Within-island speciation	14	island habitat diversity**	0.00442	0.00121	0.00332
	Adjusted $R^2 = 0.487$**					
Hawaiian	Total species richness	8	island area**	5.332	0.546	9.757
Succineidae	Adjusted $R^2 = 0.989$**		island habitat diversity**	-0.0176	0.00292	-6.018
			island isolation	1.349	0.720	1.874
			island age	-0.778	0.372	-2.089
	Within-island speciation	8	island area*	0.633	0.184	3.441
	Adjusted $R^2 = 0.608$*					
Hawaiian	Total species richness	8	island habitat diversity†	0.0814	0.0353	2.308
Achatinellinae	Adjusted $R^2 = 0.330$		island isolation	-10.369	7.164	-1.447
	Between-island speciation	8	island habitat diversity*	0.00603	0.00192	3.144
	Adjusted $R^2 = 0.578$†		island isolation*	-1.048	0.390	-2.689
	Within-island speciation	8	island habitat diversity*	0.00536	0.00194	2.769
	Adjusted $R^2 = 0.479$†		island isolation†	-0.838	0.393	-2.134

within-island is associated with habitat shifts in this group of land snails. Island area is often related to habitat diversity (Whittaker and Fernández-Palacios 2007), but number of plant species can provide a more direct measure of habitat diversity for land snails or other animals whose ecology is directly related to plant diversity (Triantis *et al.* 2003, Triantis *et al.* 2005). Different faunal groups can differ in their responses to area and habitat diversity, so that area, habitat diversity or a combination of both have a strong effects on species richness depending on the biological traits of the different taxonomic groups. Galápagos bulimulid land snail species have adapted to the different vegetation zones, and most species are found on specific plants or defined microhabitats (Coppois and Glowacki 1983, Parent 2008). Thus adaptation to specific vegetation types apparently provides the opportunity for bulimulid snails to differentiate within-island and partition the niche space to allow species to co-occur and accumulate on a given island.

Hawaiian Succineidae

I used the phylogeny published in Holland and Cowie (2009) to quantify the relative proportion of within-island and between-island speciation events on each Hawaiian young and high island (8 islands in total). The sample size for the Hawaiian Islands is much smaller than for Galápagos (8 islands compared to 14 for the partitioned analyses), and so is the associated statistical power to detect any significant trend. Nonetheless, the strongest trends can be detected. First, a model that includes island area, habitat diversity, isolation, and age (Table 2) significantly predicts total species richness of Hawaiian succineids. Island area is a significant positive predictor of island species richness in this model, and total succineid species richness is found to significantly decrease with greater island habitat diversity. This trend is contrary to what would have been predicted if ecological differences were a major contributor of species diversification in this group.

The best model predicting species richness of Hawaiian succineids due to within-island speciation includes island area as the only explanatory variable (Table 2). Therefore, larger islands support higher within-island diversification rates. In line with the finding that habitat diversity *per se* was not an important contributor to species richness, the association of within-island diversification with island area suggests that diversification might proceed via geographical isolation in Hawaiian succineids.

Finally, maybe because of the limited species representation on the phylogeny, there is never more than one speciation event inferred to be the result of colonization on each island, so that the variation in species richness due to between-island colonization cannot be tested using standard multiple regression. I therefore tested if the probability of speciation due to between-island colonization was associated with any of the biogeographical variables used in previous analyses. I find that the probability of an island to harbor a species resulting from a colonization event is significantly greater on younger islands (logistic regression coefficient =

-3.039; $z = -2.039$, $P = 0.041$). This surprising finding suggests that younger islands are more likely to harbor species resulting from between-island colonization. Younger oceanic islands tend also to be generally larger (although the association between-island area and island age is not significant), but in the case of the succineids island area per se was not found to be a significant predictor of the probability of colonization.

Hawaiian Achatinellinae

Although the multiple regression analyses for the Hawaiian Achatinellinae did not generate any model explaining a significant proportion of the variation in species richness, whether total or partitioned, the results present some interesting trends. The variation in species richness due to between-island colonization suggests that less isolated islands and islands with greater habitat diversity harbor more species. In addition, islands with greater habitat diversity had more species resulting from within-island diversification. Finally, within-island diversification rate tends to be lower on more isolated islands. Although not significant, the latter trend is expected since more isolated islands generally support less habitat diversity, and therefore provide lower ecological opportunity for diversification within-island.

DISCUSSION AND CONCLUSIONS

Together, the results of these case studies emphasize the importance of considering both speciation due to between-island colonization and within-island speciation processes in parallel when trying to determine what factors contribute to species diversity. The relative contribution of speciation due to between-island colonization and within-island speciation to total island species richness remains to be specifically quantified for larger (geographical, temporal, and taxonomical) scale studies, but this first comparative study reveals that species richness is not driven by the same processes, even in taxonomic groups that are biologically very similar.

The relative magnitudes of within-island speciation and speciation due to between-island colonization differed among the studies presented here: whereas only about 38% of island species richness of bulimulids and 40% Hawaiian succineids was inferred to be the result of the process of within-island speciation, 70% of the Hawaiian Achatinellinae represented on the available phylogeny are the result of this evolutionary process. The Galápagos snail study reveals that island area and insularity are significant correlates of speciation due to between-island colonization, whereas island habitat heterogeneity is the only significant correlate of within-island speciation. In succineids, island area is a significant predictor of both total species richness and within-island diversification rate. This highlights a potentially important difference between these two groups of species: whereas bulimulids have clearly adapted to the diverse habitats found on Galápagos and this adaptation might be associated with diversification in this group, the potential for geographical isolation might be a more important contributor to species diversification in Hawaiian succineids. With their thicker and heavier shells, bulimulids are likely to be poorer disperser than succineids. It is possible that this lower dispersal ability has allowed for the local adaptation, phenotypic evolution, and habitat associated within-island speciation that characterize Galápagos bulimulids, whereas the higher dispersal ability of Hawaiian succineids might have damped their potential for phenotypic diversification along environmental variation. However, for the succineids, within-island speciation might become possible on islands large enough to support geographically isolated populations.

The case study of the Hawaiian Achatinellinae highlights some of the limitations of the approach presented here. First, although the method seems robust (in the sense that it generates significant trends in the cases both of the Galápagos bulimulids and the Hawaiian succineids despite the incomplete sampling of these groups), it is possible that the limited species representation on the Hawaiian Achatinellinae phylogeny makes it difficult to detect any significant models. Unfortunately, given the high extinction that has devastated the Hawaiian Achatinellinae, their phylogeny will most likely always suffer from poor taxon sampling and limit the potential for inference of diversification patterns. It would be possible to test the robustness of the presented method by simulating phylogenies with various degrees of taxon sampling and analyzing the resulting trends. Second, while the diversification processes are quantified based on parsimony, and, as explained in the methods section, it is possible that the inference of speciation and colonization events does not correspond to reality. I am currently developing a maximum likelihood method to quantify the rates of *in situ* and between-island speciation, which will more fully use of the information from the phylogeny to hopefully generate estimates of the rate of these processes closer to reality. Third, the method presented here assumes that the phylogeny is the true reflection of the diversification of a lineage (i.e. there are no uncertainty at the nodes where speciation events occur). This assumption disregards the available nodal support (in the form of bootstrap replicate numbers, Bayesian probabilities or others), and the method could therefore be extended to take into account the uncertainty associated with each splitting event. Finally, not a shortcoming of the method, but the possibility remains that the diversification in the Hawaiian Achatinellinae is simply not driven by any ecological and biogeographical correlates tested here.

The results presented in this paper demonstrate that the partitioning of island species richness among diversification processes is crucial if we are to test for factors that are important in determining species diversity across biogeographical contexts and taxonomic groups. The framework I propose therefore extends the classical theory of island biogeography in two significant ways. First, by adding the evolutionary origin of species to overall island species richness, it becomes possible to disentangle the roles of ecological and evolutionary processes and their biogeographical correlates in determining species diversity. MacArthur and Wilson's theory assumed that species were neutral, that is species differences did not influence species diversity equilibrium. By considering the attributes of species (such as morphology, dispersal ability, ecological requirements, etc.) forming island communities, this framework will potentially lead to novel insights as to how species traits might be important determinants of community assembly. Because it is suitable for any island-like context, this framework combining phylogenetic and geographic information can be applied to a very broad range of systems, and will lead to a better understanding of what determines species diversity in any natural setting. An important motivation here is that humans are very rapidly transforming the natural world into island-like systems, such that processes involved in determining island biodiversity will become increasingly relevant to global biodiversity. A better understanding of ecological and evolutionary processes on island systems will offer insight into the long-term prospect for global biodiversity. The framework presented here has therefore broad implications for the study of species diversity in general since such process partitioning is likely relevant to many island-like systems and fragmented habitats; such systems are becoming the norm in our modern world.

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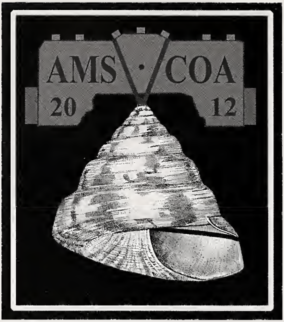

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